PB85-127199

Recovery of Trace Organic Compounds by the Parfait/Distillation Method

Illinois Univ. at Urbana-Champaign

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

Nov 84

US Department of Commerce
National Technical Information Service
NTIS

PB85-127199

EPA-600/1-84-013
November 1984

RECOVERY OF TRACE ORGANIC COMPOUNDS BY THE PARFAIT/DISTILLATION METHOD

by

James B. Johnston
Clarence Josefson
Richard Trubey

Institute for Environmental Studies
University of Illinois
Urbana, IL 61801

Cooperative Agreement CR 807126-01

Project Officer

Frederick Kopfler
Toxicology and Microbiology Division
Health Effects Research Laboratory
Cincinnati Ohio 45268

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NORTH CAROLINA 27711

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/1-84-013		PB85-127199

4. TITLE AND SUBTITLE	5. REPORT DATE
Recovery of Trace Organic Compounds by the Parfait/Distillation Method	November 1984
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
James B. Johnston, Clarence Josefson, Richard Trubey	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Institute for Environmental Studies University of Illinois Urbana, IL 61801	CAYB1B
	11. CONTRACT/GRANT NO.
	Cooperative Agreement CR807126-01
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711	Final; Jan. 1980 - July 1983
	14. SPONSORING AGENCY CODE
	EPA-600/11

15. SUPPLEMENTARY NOTES
Project Officer: Frederick C. Kopfler, TMD, HERL

16. ABSTRACT

A modified parfait/distillation method was developed that recovers a wide range of neutral, catonic, anionic, and hydrophobic contaminants form water. Porous polytetrafluoroethylene (PTFE, Teflon, duPont) was identified as an ideal first filtering-adsorbing bed in the parfait train. PTFE removed humic acid and a broad range of hydrophobic compounds. It was more easily cleaned and contributed fewer impurities to eluates than other porous hydrophobic adsorbents tested. Several types of 0.2 μm sterilization filters were tested. Filters containing nitrocellulose or fiberglass appeared to adsorb appreciable amounts of some model compounds; polycarbonate or polypropylene filters were less adsorptive. Various ion exchange resins were evaluated for use in the parfait column. The resins selected, Dowex MSC-1 and Duolite A-162, carried strongly acidic or basic exchange groups, were macroporous, had relatively high exchange capacities, and were relatively inexpensive. An elution protocol was developed, with emphasis on recovery of poorly volatile, high water-soluble model compounds. Finally, the modified parfait method was tested for its ability to recover 19 model compounds in a synthetic hard water. Poorly volatile, neutral, water-soluble contaminants (hexoses) and cationic aromatic compounds were recovered with exceptional efficiency. Humic acid was readily recovered and did not substantially interfere with the recovery of the other model compounds. Model amphoteric compounds were removed from water, but were difficult to recover from the parfait column. Trimesic acid was readily removed from water and was recovered selectively, though not quantitatively.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to Public	unclassified	81
	20. SECURITY CLASS *(This page)*	22. PRICE
	unclassified	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

NOTICE

This document has been subjected to the U.S. Environmental Protection Agency's peer and administrative review policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides, and non-ionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

The Health Effects Research Laboratory has been charged with evaluating preconcentration methods for the analysis of waters. Eventually, the Laboratory hopes to use such methods in experiments to develop sound estimates of the health risks from contaminants of waters. The development and characterization of the parfait methods reported here was undertaken jointly with the Health Effects Research Laboratory.

F. Gordon Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

The parfait/distillation method uses a sequential series of adsorbents to remove contaminants from water and vacuum distillation to recover unadsorbed materials. A modified parfait method was developed that recovers a wide range of neutral, cationic, anionic, and hydrophobic contaminants from water. Porous polytetrafluoroethylene (PTFE, Teflon, duPont) was identitied as an ideal first filtering-absorbing bed in the parfait train. PTFE removed humic acid and a broad range of hydrophobic compounds. It was more easily cleaned and contributed fewer impurities to eluates than other porous hydrophobic adsorbents tested. Several types of 0.2-μm sterilization filters were tested. Filters containing nitrocellulose or fiberglass appeared to adsorb appreciable amounts of some model compounds; polycarbonate or polypropylene filters were less adsorptive. Various ion exchange resins were evaluated for use in the parfait column. The resins selected, Dowex MSC-1 (Sigma) and Duolite A-162 (Diamond Shamrock), carried strongly acidic or basic exchange groups, were macroporous, had relatively high exchange capacities, and were relatively inexpensive. An elution protocol was developed, with emphasis on recovery of poorly volatile, highly water-soluble model compounds. Finally, the modified parfait method was tested for its ability to recover 19 model compounds in a synthetic hard water. Poorly volatile, neutral, water-soluble contaminants (hexoses) and cationic aromatic compounds were recovered with exceptional efficiency. Humic acid was readily recovered and did not substantially interfere with the recovery of the other model compounds. Model amphoteric compounds were removed from water but were difficult to recover from the parfait column. Trimesic acid was readily removed from water and was recovered selectively, though not quantitatively.

The modified parfait system should be most useful in exploratory surveys of poorly volatile organic contaminants in water and in studies to detect water-soluble mutagens.

ACKNOWLEDGEMENT

Preparation of the scanning electron micrographs by Ms. Manroop Chawla is gratefully acknowledged.

CONTENTS

Page

Foreword iii

Abstract iv

Acknowledgement v

1. Introduction 1
2. Conclusions 3
3. Recommendations 5
4. Materials and Methods 7
   - General considerations 7
   - Reagents 9
   - Materials 9
   - Instrumentation 10
   - Basic procedures 10
   - Analytical methods 12
   - The modified parfait protocol 13
5. Results and Discussion 18
   - Losses on ultrafilters 18
   - Losses during vacuum distillation and lyophilization 18
   - Alternatives to silica gel as the first parfait bed 21
   - Characterization of porous Teflon 27
   - Ion exchange resins 40
   - Humic acid 54
   - Recovery of test solutes 55
   - Future use of the parfait method 67

References 69

Preceding page blank

SECTION 1

INTRODUCTION

The need to evaluate health risks associated with organic contaminants in surface and potable waters has prompted basic research into methods to recover and concentrate these substances. The contaminants about which most is known are hydrophobic or volatile substances, which are readily recovered by methods such as gas stripping and solvent extraction. However these compounds account for only a minority of the organic material in a typical water (Shackelford and Keith, 1976; Rosen, 1977). The majority of compounds in water are poorly understood, both because methods to recover them have not been developed and because automated means of compound identification, such as gas chromatography/mass spectrometry (GC/MS), are not applicable.

Before the work reported here was begun, we attempted, in an exploratory study, to recover the soluble, poorly volatile subclass of organic compounds from water. We used a set of adsorbents (1) to filter particulate matter and to adsorb hydrophobic compounds, (2) to recover cations and amphoterics on a cation exchange bed, and (3) to recover anions on an anion exchange bed. The effluent from this series of adsorbents contained the neutral compounds. The eluates from each bed and the effluent were then concentrated under vacuum. This system, the parfait method, was demonstrated to recover parts-per-billion concentrations of several known mutagens in amounts sufficient to be detected by bioassay (Johnston and Verdeyen, 1981). The studies developing the parfait system were formulated with the aim of recovering and detecting mutagenic substances, because, in principle, these constitute the main toxicological hazard associated with highly dilute contaminants in water. The parfait method also has been proposed as a means to preconcentrate contaminants for qualitative and quantitative chemical analysis. However, such use of the method would depend upon a much more thorough characterization of the kinds and amounts of compounds that it recovered and, in addition, further development of the method to overcome certain inherent problems.

The original parfait method rested on the use of vacuum distillation/lyophilization to concentrate the poorly volatile species in water. It might be expected that the removal of water under vacuum should be simple and straightforward. Vacuum distillation and

lyophilization does indeed recover the poorly volatile contaminants from unfractionated surface waters. However, the compounds are obtained in an intractable, insoluble form, because bicarbonate dissociates under vacuum to give metal carbonate precipitates that can trap organic polymers and lipids (Otsuki and Wetzel, 1973; Suess, 1970). The parfait method prevents the formation of precipitates by removing metal ions on a strongly acidic cation exchange bed.

To protect the bed from particulate matter and to remove some of the hydrophobic species, the original parfait system used a bed of silica gel placed ahead of the cation exchange bed. To neutralize the acid released from the cation bed, as well as to remove anions, a strongly basic anion exchange bed was added after the cation exchanger. The silica gel created problems in the original parfait system because it acted as a weak cation exchanger and because it leached silicic acid to the anion exchange bed and into the final effluent. The silicate residue interfered with the recovery of compounds from these two sources.

In the first part of the study reported here, we identified porous polytetrafluoroethylene (PTFE; Teflon, a registered trademark or E.I. dupont de Nemours and Co.) as a suitable substitute for silica gel as the first filtering-adsorbing bed in the parfait train. Two porous Teflons, Chromosorb T and Fluoropak 80, were tested. Chromosorb T was preferred. Next, we developed a protocol to selectively desorb organic anions and cations from the ion exchange beds. Finally, we evaluated the model system by recovering 19 model compounds added at 50 ppb to a synthetic hard water. The model compounds were analyzed quantitatively in the presence or absence of 2 ppm humic acid.

SECTION 2

CONCLUSIONS

A modified parfait method was developed that is capable of recovering a wide range of neutral, cationic, anionic, and hydrophobic contaminants from water. This method quantitatively recovered neutral, poorly volatile, highly water-soluble compounds such as glucose. It may be the best available method for recovering such contaminants.

The success of the modified parfait method depends heavily upon the properties of porous PTFE. This material removed humic acids and a broad range of poorly water-soluble compounds from water. It also had an anomalously high affinity for cationic aromatic compounds, which it adsorbed quantitatively. Porous PTFE showed an inverse correlation of capacity factor and aqueous solubility for a series of hydrocarbons. It displayed an anomalous direct correlation of capacity factor and solubility for a series of alkyl-substituted xanthines. Porous PTFE was more easily cleaned and contributed fewer impurities to eluates than porous hydrophobic adsorbents such as polystyrene-divinylbenzene polymers. PTFE may be a highly desirable alternative to other currently available porous hydrophobic resins, particularly in studies concerned with the quantitative analysis or the qualitative enumeration of hydrophobic organic contaminants in water.

The modified parfait system removed model amphoteric compounds from water, but they were difficult to recover from the parfait column by the protocols developed for weak acids and bases. Amphoteric compounds were not recovered quantitatively from the adsorbent beds. The parfait method is therefore not particularly well suited for the recovery of these compounds, and further work would be needed to adapt it to this purpose. Trimesic acid was readily removed from water by the parfait method and could be selectively recovered from the anion exchange resin. Recoveries were not quantitative; however, the selective recovery of polyanions should greatly facilitate their subsequent isolation and analysis.

Several hydrophobic compounds were shown to be readily lost from water during vacuum distillation and during freeze drying. Compounds like biphenyl were lost in substantial amounts even in the brief initial degassing phase of vacuum distillation. Compounds at least as volatile

as pyrene probably cannot be reliably concentrated from water by vacuum distillation. This behavior was of little consequence, however, to recovery of such compounds by the modified parfait system. Hydrophobic materials were recovered in the first parfait bed by adsorption onto PTFE. Materials from this bed were eluted in methylene chloride and concentrated by solvent evaporation in a Kuderna-Danish flask under a three-ball Snyder column. Under these conditions, recoveries were satisfactory.

The humic acid used in this study was readily recovered by the parfait method and did not substantially interfere with recovery of the other model compounds, even when humate was present initially at parts-per-million concentrations.

Certain disposable 0.2-μm (pore size) sterilization filters adsorbed appreciable amounts of some of the model compounds from water. Filters containing fiberglass or nitrocellulose adsorbed more of the solutes tested than those composed only of polycarbonate or polypropylene; they did not allow quantitative recoveries of contaminants. This consideration is particularly important when ultrafiltration is to be used in the unobtrusive sterilization of an aqueous sample, for instance, for mutagenicity assay.

SECTION 3

RECOMMENDATIONS

Porous PTFE exhibits high affinity for a broad range of hydrophobic compounds, humic acids, and positively charged aromatic compounds, whereas solid PTFE of similar total surface area but a smaller internal (void) volume does not. The basis for porous PTFE's adsorptivity needs to be learned.

Porous PTFE should be characterized as a replacement for porous hydrophobic resins of the polystyrene-divinylbenzene or polyacrylate type. It is more inert, more stable, and contributes fewer impurities to eluates than these other resins. Porous PTFE has a capacity for hydrocarbons similar to XAD-8. It recovers a similar, but not identical, spectrum of compounds from water. A detailed comparison of porous PTFE and the resins currently in use should be carried out.

PTFE's exceptional recovery of humic acids should be studied with a variety of humic and fulvic acids to assess the generality of the adsorption and to determine whether PTFE could be used for the recovery, fractionation, and characterization of humic substances.

The parfait system should be employed in conjunction with short-term toxicological assays to survey typical drinking water supplies for mutagens and other toxicants. It should also be used with chemical assays when the recovery of neutral, water-soluble, or cationic aromatic compounds is to be emphasized. The method does not recover highly volatile compounds and it does contribute resin contaminants to eluates of the ion exchange beds. Nevertheless, it is very useful for the recovery of a broad range of poorly volatile organic contaminants and should find use in exploratory surveys of these contaminants in aqueous media. It should be particularly useful in studies to detect water-soluble mutagens.

The recovery of amphoteric compounds from parfait adsorbents is poor. Further study and modification of the parfait method is needed before these compounds can be quantitatively recovered by this method. Study of these contaminants will require either further modification of the parfait protocol to enhance the recovery of amphoterics from parfait adsorbents or a method specifically designed to recover amphoterics.

The parfait method is not suited to the recovery of volatile contaminants in water and should not be used for this purpose.

SECTION 4

MATERIALS AND METHODS

GENERAL CONSIDERATIONS

In this study, we investigated and attempted to correct the deficiencies of the original parfait method and then assessed the quantitative recovery of the 19 model compounds listed in Table 1, spiked into a synthetic hard water at the concentrations listed. Recoveries were assessed in the presence and absence of 2 ppm of a humic acid supplied by the U.S. Environmental Protection Agency (EPA) Health Effects Research Laboratory (HERL). Finally, the recovery of 16 of 19 solutes from a single mixture including humic acid was determined.

Whenever possible, the simplest, most readily available analytical methods were used in this study, to ensure that the thrust of the study was the development of the recovery method, and not the study of the problems inherent in the simultaneous analysis of 16 compounds in the presence of humic acids. The emphasis of the study was not to achieve high analytical precision or to carry out large numbers of replicate experiments, but rather to improve the recovery method itself. To find the best overall protocol for recovery of the model compounds, many variations in protocol were tried. The analytical methods used were adequate to discern the superiority of a new protocol but were not always the most precise methods possible. Because the model compounds in this study represented general classes of environmental compounds, we felt that the recovery of any model compound in a synthetic hard water to a precision of two significant figures would be sufficient to indicate the probable utility of the method for recovering similar environmental compounds of interest.

During the study, several factors were found to limit reproducibility. The overall reproducibility of compound recoveries was limited in some cases by the reproducibility of the analysis of individual compounds, as demonstrated in a limited number of trials. In most experiments, the large number of manipulations involved in the parfait method also contributed to variance in compound recovery. In some series of experiments, variance appeared to decrease as experience with the manipulations and analyses increased.

TABLE 1. COMPOUNDS USED TO ASSESS THE PERFORMANCE OF THE PARFAIT RECOVERY METHOD

Compounds	Concentration (μg/l)
Quinaldic acid	50
Trimesic acid	50
Stearic acid	50
Glycine	50
Furfural	50
Quinoline	50
Caffeine	50
5-Chlorouracil	50
Glucose	50
2,4'-Dichlorobiphenyl	50
2,2',5,5'-Tetrachlorobiphenyl	50
bis(2-Ethylhexyl)phthalate	50
1-Chlorododecane	50
Biphenyl	50
Isophorone	50
Anthraquinone	50
Methylisobutylketone	50
2,4-Dichlorophenol	50
Phenanthrene	1
Humic acid	2000

We decided at the outset to focus on the anticipated strength of the parfait method: the recovery of the poorly volatile, highly water-soluble model compounds. No effort was made to evaluate recovery of very volatile compounds such as chloroform, which would be lost in any version of the parfait method. While developing the protocol, we assayed fractions selectively, so that preparation and assay of the most time-consuming fraction, the parfait column effluent, was not carried out in the early experiments with single compounds unless a compound could not be adequately accounted for in the other fractions (i.e., experiments with hydrophobic or strongly ionized compounds, which did not enter the column effluent).

In the final demonstration runs, 16 compounds plus humic acid were tested simultaneously. Three other compounds (quinaldic acid, 5-chlorouracil and phenanthrene) were run and assayed as a separate group, because one or more of the first 16 compounds interfered with their analysis.

In sequential recovery processes, the first step often strongly influences the efficiency of the later steps and may actually dictate the choice of the later steps. In the original parfait method for instance, the silica gel interfered with the cation bed by acting as a weak cation exchanger and by potentially adsorbing some, but not all, hydrophobic species. It also interfered with the anion exchange bed and the column effluent by releasing silicic acid to them. Usually, the first step would be expected to influence later steps primarily by adsorbing or not adsorbing particular components of the mixture of contaminants. Careful choice and evaluation of the first step in an adsorption train is therefore very important; accordingly, this project was guided by the principle that selection of the early recovery steps deserved greater effort than selection of the later steps.

REAGENTS

Sigma Chemical Co. supplied tryptophan, quinaldic acid, trimesic acid, glycine, caffeine, 5-chlorouracil, xanthine, uracil, ethidium bromide (EB), theophylline, theobromine, 7(2,3-dihydroxypropyl)theophylline, fluorescamine, isobutyl-1-methylxanthine, dextran blue, and benzylamine. Analabs, Inc., supplied stearic acid, 2,4′-dichlorobiphenyl, and 2,2′,5,5′-tetrachlorobiphenyl. Eastman supplied phenanthrene. Aldrich supplied pyrene, fluoranthene, furfural, indole, quinoline, glucose, bis(2-ethylhexyl)phthalate, biphenyl, acridine, isophorone, anthraquinone, methyl isobutyl ketone, 9-methylanthracene, 2,4-dichlorophenol, 2,6-di-tert-butyl-4-methylphenol (BHT), 1-naphthol, adenine, 3-methylxanthine, 7(2-chloroethyl)theophylline, acenaphthene, and 2,2′-biquinoline. Fluka supplied 1-chlorododecane and produced the humic acid supplied by HERL. Inorganic reagents were supplied by Baker. All reagents were of the highest purity supplied by the manufacturers and were used without purification. High-purity water was obtained by passing deionized tap water through a Millipore MilliQ apparatus containing an activated carbon cartridge, a high-capacity, mixed bed ion exchange cartridge and an ultra-pure ion exchange cartridge. Water exited the apparatus through a 0.2 μm ultrafilter. Hexane, acetone, dichloromethane, and methanol were Burdick and Jackson distilled-in-glass.

MATERIALS

Silica gel 60 (70-230 mesh) was obtained from Brinkman; florisil (100-200 mesh) was from Fisher Scientific; cellulose powder was from Whatman; glass wool and glass beads (60-80 mesh) treated with dichlorodimethylsilane were from Alltech Associates. Untreated glass beads (80-120 mesh) were supplied by Applied Science Labs; porous Teflon (Chromosorb T, 30-60 mesh, 7-8 m^2/g total surface area) was manufactured by Johns-Manville and supplied by Alltech Associates. Fluoropak 80 Teflon particles (40-60 mesh, 2-4 m^2/g total surface area) were supplied

by the Fluorocarbon Co. Amberlite resins IR 120, IR 252, IRA 400, and IRA 900 were supplied by Mallinckrodt; Amberlite XAD-2 and XAD-8 were supplied by Chemical Dynamics; Diamond Shamrock supplied Duolite resins A-7, A-162, and S-587; Sigma supplied Dowex MSC-1; and BioRad supplied AG MP-50 and AG MP-1 resins. The characteristics of each resin and the cleanup procedures used for each are described in Section 5, Results and Discussion.

INSTRUMENTATION

Electron micrographs of platinum-shadowed Teflon particles were made with an International Scientific Instrument model DS130 scanning electron microscope. Absorption of parfait column eluates was measured in a Beckman Acta III or a Perkin-Elmer Model 552A UV-visible spectrophotometer. Fluorescence was measured in a Perkin-Elmer MFP-44B spectrofluorimeter. In the Teflon breakthrough studies, column fractions were collected by an ISCO model 1850 fraction collector equipped with a drop counter. A Varian 2700 gas chromatograph with flame ionization detector (FID) was used to quantify volatile solutes and the volatile methyl esters of the poorly volatile acidic solutes. Vacuum distillation of parfait column effluents was performed on an FTS Systems model FD-20-84 high-capacity, corrosion-resistant freeze drying apparatus. Some concentrates were analyzed on a Hewlett-Packard model 5985 gas chromatograhic/quadrapole mass spectrometer.

BASIC PROCEDURES

The synthetic hard water used in all of the parfait column studies and for most of the Teflon adsorption studies consisted of 0.070 g $NaHCO_3$, 0.156 g $CaSO_4 \cdot 2H_2O$ and 0.047 g $CaCl_2$ per liter. This solution was prepared in 8-l batches by dissolving the freshly weighed solids into high-purity water just before use. The final pH of the solution was 7.2. This medium is representative of the inorganic load encountered in typical potable waters.

For parfait column studies, standard solutions (400 µg/ml) of each organic solute except phenanthrene were prepared in a suitable solvent (acetone, water, or methanol), and 1.00 ml of the standard was added to 8 l of the synthetic hard water with rapid stirring. This yielded a final organic solute concentration of 50 µg/l and a solvent concentration of less than 126 mg/l. In the runs simultaneously involving 16 compounds, four different mixtures of solutes were prepared so that the final solvent concentration was 500 mg/l. Phenanthrene was spiked to a final concentration of 1.0 µg/l, rather than 50 µg/l. Solutions were not degassed before application to a parfait column. Humic acid was dissolved and added to synthetic hard water as indicated in the subsection entitled Humic Acid, in Results and Discussion. No adjustments of pH were necessary after adding solutes to the synthetic hard water.

For the breakthrough studies with Teflon, stock solutions of the poorly water-soluble compounds pyrene, fluoranthene, fluorene, acenaphthene, phenanthrene, 9-methylanthracene, acridine, 2,2′-biquinoline, and biphenyl were prepared by a modification of the "generator column" method (May et al., 1978). About 10 ml of a 1% solution of the solute in hexane was poured over 15 g of 80-120 mesh glass beads in a beaker, and the solvent allowed to evaporate in a fume hood, with occasional stirring. In this manner, the beads were coated with a thin film of the solute. The coated beads were transferred to a BioRad 1.4-cm-i.d. glass chromatography column and the synthetic hard water was passed over the beads at about 300 ml/h. The concentration of a solution prepared in this manner was determined by comparing the fluorescence of an aliquot diluted 20% with methanol with that of a set of standards prepared by dissolving the solid reagent directly into 20% methanol in the synthetic hard water. Table 2 lists the wavelengths used for analysis, the concentrations of these stock solutions, and a literature value for the solubility of each solute. The generator column method is a convenient and rapid means to prepare solutions of hydrophobic compounds at or just below saturation.

Columns for breakthrough studies consisted of five-ml beds of Chromosorb T (2.65 g) or Fluoropak 80 (3.70 g) prepared by suspending each resin in acetone and pouring the slurry into BioRad 0.90-cm-i.d. glass chromatography columns equipped with stainless steel stopcocks. The acetone level was brought just to the top of the column packing and glass-distilled, deionized water was carefully loaded into the column in small increments. Columns were washed with a minmum of 100 bed volumes of water and used within 24 hours. Before constructing a breakthrough curve, a column was washed with 100 bed volumes of the synthetic hard water, and fractions collected when the solute solution was started as the influent; flow was 1.7 ml/min. A maximum of 1400 bed volumes of solution were applied to any column.

TABLE 2. STOCK SOLUTIONS PREPARED USING GLASS BEAD GENERATOR COLUMNS

Solute	Absorption or Fluorescence		Concentration (mg/l)	
	ex (nm)	em (nm)	stock	solubility[a]
Pyrene	330	375	0.14	0.135
Fluoranthrene	354	464	0.17	0.26
9-Methylanthracene	282	410	0.25	0.261
Phenanthrene	294	365	0.82	1.29
Acenaphthene	291	-	2.5	3.93
Biphenyl	248	-	6.6	7.0
5-Chlorouracil	270	-	15	-
Acridine	357	425	23.4	-

[a] From MacKay et al., 1980.

ANALYTICAL METHODS

The determination of void volumes took advantage of Teflon's hydrophobicity. Columns were packed and washed as usual, and the water column was brought just to the top of the bed. The assembled column was weighed, the packing expelled by air into a tared beaker and the packing plus the column were dried overnight at 70°C. The void volume was taken as the water lost on drying, determined after reweighing the dry column components.

An alternative method was used to verify the accuracy of the V_o determinations. After the water level was brought to the top of a packed, washed bed, tritiated water, 1 μCi/ml, was loaded into the column. Two-drop fractions were collected by hand into tared scintillation vials and the vials reweighed to determine the collected volume. After adding 5 ml of Packard Monophase 40 scintillation cocktail, the tritium was counted. A plot of specific activity versus eluted volume was constructed and V_o was taken as the volume at which the effluent had 50% the specific activity of the influent. Values of V_o were corrected by substracting the dead volume of the column, i.e., the volume contained in the stopcock and in the column below the scintered glass support of the column packing.

For the breakthrough studies with Teflon, the concentration of solute in the column effluent was estimated by comparing absorbance or fluorescence of the unknown with standard curves for dilutions of the same stock solution used to prepare the column influent. These results are expressed as the ratio of solute concentrations in effluent and influent, c/c_o.

Gas chromatography with a flame ionization detector was used to separate and quantify all solutes evaluated on parfait columns except glycine, glucose, phenanthrene, and 5-chlorouracil. Acidic compounds were methylated prior to chromatography with at least a 100-fold molar excess of diazomethane in diethyl ether, prepared from Diazald using the Aldrich procedures. Materials recovered from Teflon in early experiments were chromatographed in a 2-mm-i.d. x 1.8-m silanized glass column containing 3% OV-101 on 80-100 mesh Supelcoport. The conditions for chromatography were as follows: detector, 230°C; injection port, 200°C; column, 100° to 250°C at 10°/min. In later work (column numbers greater than 93), 3% Carbowax 20M on 60-80 mesh acid-washed, dichlorodimethylsilane-treated Chromosorb W replaced the OV-101 packing. The Carbowax packing is insensitive to water. In fact, direct injection of the vacuum distillates of the parfait column effluent was used to detect isophorone and caffeine in fraction 7 (see below). Generating conditions for chromatography on Carbowax were as follows: detector 220°C; injection port, 210°C; column, 100° to 200°C at 8°/min. The principal difficulty with the Carbowax packing was that it could not be used at temperatures above 200°C. All gas chromatography was initialized to a nitrogen carrier gas flow rate of 30 ml/min at 100°C. Columns were frequently repacked, especially if there was noticeable deterioration in peak resolution.

In typical experiments, 4-µl aliquots of concentrates were injected. Recoveries were determined from the areas of peaks, and each determination was calibrated with standards prepared from the same stock solutions used to spike the column influent. If the identity of a peak determined by retention time alone was uncertain, that extract was spiked with a known amount of standard and rechromatographed to identify the compound and to enable an internal calibration of its amount. The standards for acidic solutes were always methylated at the same time as each experimental concentrate. Reconstruction experiments showed that methylation efficiency was the same in the solvent used to prepare the standard and that obtained from experimental parfait column concentrates. Chromatography was usually completed within 36 h of final concentration or methylation of a sample. Samples were stored in Teflon-lined crimp-sealed vials at -20°C.

Glycine was analyzed by the fluorescence of its fluorescamine derivative (ex = 366 nm, em = 480 nm; Coppola and Hanna, 1976). A standard working curve prepared simultaneously with the analyte permitted quantitation.

Glucose was analyzed by the glucose oxidase method (Sigma Chemical Co., 1979; Fales, 1963; Washko and Rice, 1959) using reagents obtained from Sigma. Glucose was enzymatically oxidized to gluconic acid with the production of hydrogen peroxide. The peroxide was reacted with o-dianisidine to produce a red chromophore. Unknowns were quantified by comparing absorbances at 500 nm with those of standards prepared at the same time.

Phenanthrene was analyzed directly by fluorescence, ex = 365 nm, em = 294 nm, and quantified by comparison to a standard curve; 5-chlorouracil was analyzed directly by absorbance at 270 nm. Attempts to form derivatives of 5-chlorouracil by pyrolysis of its tetramethylammonium salt are discussed below; however, this method gave unsatisfactory results. Hypochlorite was determined by the ferrous-DPD titration method (Franson, 1976).

THE MODIFIED PARFAIT PROTOCOL

The development of the modified parfait protocol is described in Section 5, Results and Discussion. The procedure finally chosen for cleaning the adsorbents and recovering the model solutes is given here.

Teflon adsorbents were cleaned by 24-h extraction with acetone in a Soxhlet apparatus and stored in acetone. Cleaned Chromosorb T was used to fill a 150-ml silanized addition funnel equipped with a 24/40 standard taper female joint on top and a Teflon stopcock and a 24/40 male joint on the bottom. A plug of silanized glass wool supported the bed just above the stopcock. The Teflon bed was compressed slightly with a large stirring rod to keep the top of the bed in place while the bed was washed with at least six bed volumes (900 ml) of high-purity water. The washed Teflon was kept covered with water and used immediately.

Large quantities of ion exchange resin MSC-1, a strongly acidic macroporous cation exchange resin, and Duolite A-162, a strongly basic macroporous anion exchange resin, were cleaned at one time. A resin to be cleaned was packed into a 3-l capacity addition funnel and washed first with two volumes of high purity water. The MSC-1 resin was next washed sequentially with two bed volumes of 2.0 N HCl, five bed volumes of high-purity water, two bed volumes of 1.5 N NaOH, and five bed volumes of high-purity water; these HCl, water, NaOH, and water washes were then repeated. The same procedure was carried out with A-162, except that the order of acid and base was reversed. The resins were then extracted for at least 16 h in methanol in a Soxhlet apparatus. The final cleanup steps for MSC-1 consisted of a sequential elution with two bed volumes of methanol, one bed volume of 1.0 N NH_3 in 12% methanol/88% methylene chloride (made as described below), two bed volumes of 12% methanol/88% methylene chloride (solvent 1), and three bed volumes of methanol. The same steps were used with A-162 except that HCl replaced NH_3 in the second wash. The resins were considered to be clean at this point, and they were stored in methanol.

The NH_3 or HCl solutions in solvent 1 were prepared by passing the anhydrous gas through the methanol/methylene chloride and protecting the solution from water vapor using anhydrous $CaSO_4$, as indicated in Figure 1.

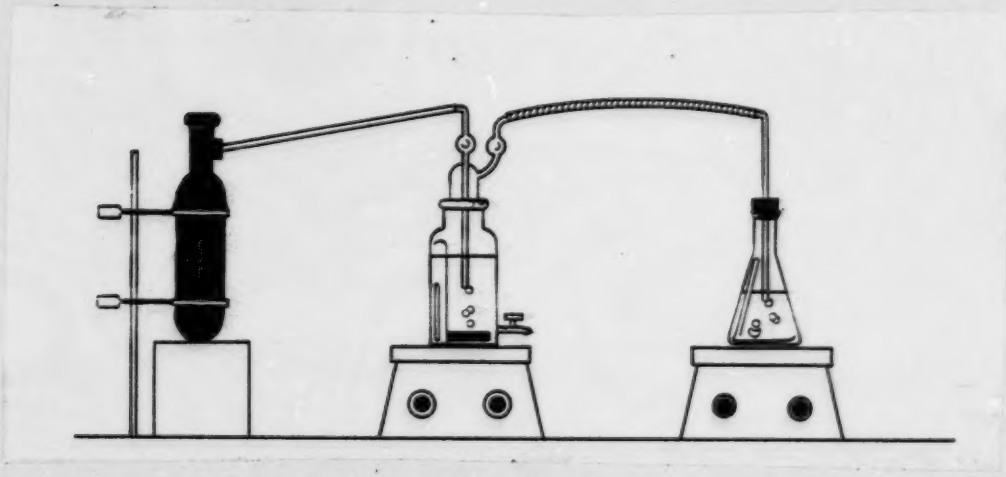

Figure 1. The apparatus used to prepare anhydrous solutions of NH_3 of HCl in solvent 1. The lecture bottle holds the anhydrous gas; the tubing connecting the gas exhaust to the trap is filled with drying agent.

The ion-exchange resins were loaded in a methanol slurry into parfait column addition funnels over a small plug of silanized glass wool. Thirty milliliters of MSC-1 was loaded into a 150-ml funnel, and 65 ml of A-162 was loaded into a 60-ml funnel, filling it and preventing the resin from floating during later elution steps. The total exchange capacity of each bed was at least 50 meq. The resins were drained of methanol, two bed volumes of high-purity water were passed over the bed, and then two bed volumes of 2.0 N HCl (MSC-1) or 1.5 N NaOH (A-162) were passed through to charge the exchange sites. This was followed by at least of five bed volumes of high-purity water, until the pH of the effluent (measured with pH paper) was near neutrality. From the initial water wash after the methanol was drained off, the beds were kept covered with solvent (i.e., were not allowed to run dry). The addition funnels were then connected to each other in order, Teflon above MSC-1 above A-162. A 10-l glass carboy containing the synthetic hard water and any test solute was put in place above the Teflon bed. A siphon flow through 4-mm-i.d. Teflon tubing was started by applying a gentle air pressure to the open hole of a two-holed stopper through which the tubing passed. The other end of the Teflon tubing passed through and beyond a one-holed rubber stopper fitted to the top of the porous Teflon bed of the parfait column. Thus, the water contacted only glass or Teflon surfaces before encountering the first parfait bed.

Flow through the column was initiated, and the column effluent was collected directly into a silanized 12-l round-bottom flask, at a nominal flow rate of 15 ml/min, adjusted with the bottom stopcock. The entire parfait set-up is illustrated in Figure 2. At the end of a run, 500 ml of high-purity water was passed through the train of adsorbents, which were allowed to drain completely by gravity.

The Teflon and ion exchange beds were eluted with the eluents indicated below as follows: the addition funnels containing each bed were separated from the parfait column and a separate, empty addition funnel was loosely mounted on top to supply eluents. The bottom stopcock was closed and the first eluent was slowly added until air had escaped the bed and the bed was covered by 0.5 cm of liquid. The upper addition funnel was then firmly seated and elution began by opening the bottom stopcock. Flow was adjusted to less than 3 ml/min with the bottom stopcock. New eluents were added to the upper addition funnel as it ran dry. The adsorbent bed in the lower funnel was kept constantly covered with 1 to 5 mm of eluent.

The Teflon bed was eluted with four bed volumes of methylene chloride, which was collected directly into a separatory funnel. The water layer that formed in the eluate was separated and discarded. This methylene chloride became parfait fraction 1 (F1).

The addition funnels containing the ion exchange beds were dried and eluted with a series of solvents. To dry each bed two bed volumes of absolute methanol was passed through, followed by two bed volumes of absolute ethanol, followed by two more bed volumes of methanol. Each

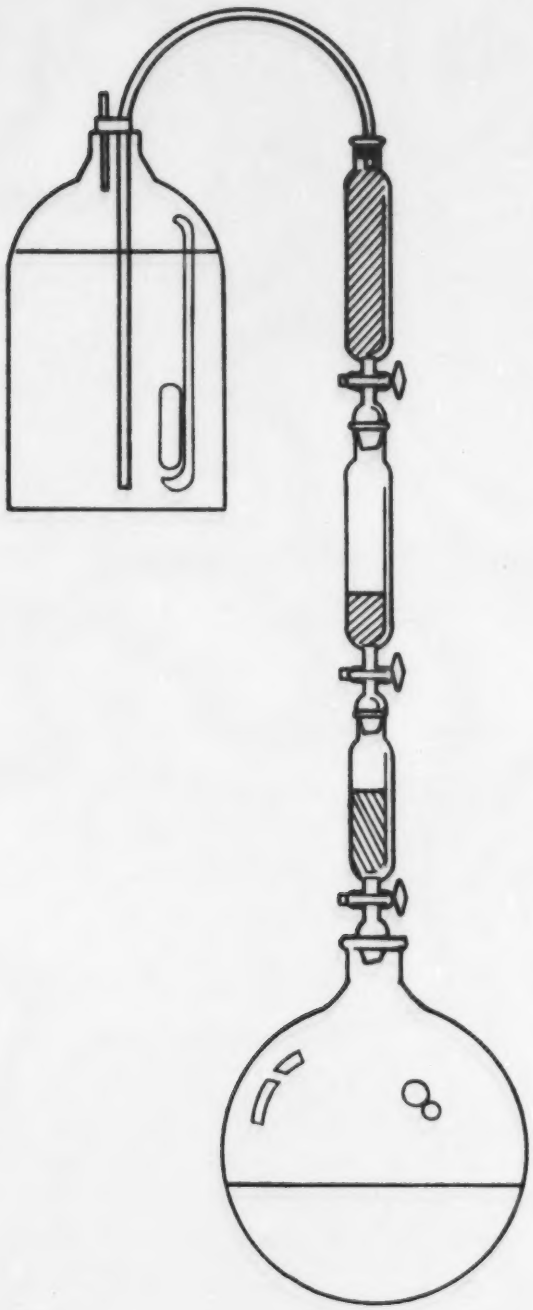

Figure 2. The modified parfait column.

bed was next washed with two bed volumes of solvent 1. This washing diminished the shock to the resins of the next solvents. The ethanol, methanol, and solvent 1 eluates were discarded.

The MSC-1 bed was eluted with 150 ml of 0.33 N NH_3 in solvent 1, four bed volumes of 0.1 N NH_3 in solvent 1, and then four bed volumes of methanol. The combined eluates became parfait fraction 2 (F2).

The A-162 bed was eluted first with 150 ml of 0.33 N HCl in solvent 1 (F3) and then with four bed volumes of 0.10 N HCl in solvent 1 (F4), four bed volumes of methanol (F5), and four bed volumes of 0.10 N HCl in methanol (F6).

Each of these six organic fractions was reduced to a volume of about 5 ml in a Kuderna-Danish concentrator under a 3-ball Snyder column and then to a volume of 1.0 ml under a gentle stream of dry nitrogen. These concentrates, or their methylated derivatives adjusted to the same concentration, were analyzed as described above.

The effluent from the parfait column was exposed to vacuum in a silanized 12-l flask on a high-capacity freeze dryer. The effluent was stirred with a magnetically coupled stirring bar and heated by immersion of the lower half of the flask in a 30°C constant-temperature water bath. The rate of distillation was controlled to about 8 1/24 h with a needle-valve air bleed. Distillation was continued to a volume of about 500 ml, when the concentrate was transferred to a smaller flask and the volume further reduced to 10 ml on a conventional rotary evaporator.

SECTION 5

RESULTS AND DISCUSSION

LOSSES ON ULTRAFILTERS

Originally, parfait concentrates were used for the detection of mutagens in water with the Ames Salmonella/microsome reversion assay. Therefore, each concentrate had to be free of contaminating bacteria that could interfere with this assay. Filtration through 0.2 μm (pore size) ultrafilters was chosen as the least intrusive means to sterilize these extracts. To test the possibility that the filters themselves could adsorb mutagens or other substances, several ultrafilters were exposed to solutions of pyrene, 0.14 μg/l in synthetic hard water, and EB, 1.0 μg/l, in high-purity water. The results are shown in Figure 3. All of the filters adsorbed significant amounts of pyrene, and the Millex disposable filter unit also adsorbed over 100 μg of EB, a considerable amount in terms of mutagenicity. Of the filter materials tested, fiberglass prefilters seemed to have an unacceptably high capacity for the solutes, and polycarbonate filters mounted in reusable polycarbonate filter holders seemed to be the most acceptable (i.e., the least adsorptive).

Although these results cannot be generalized to many compounds, they serve to point out a source of potential losses of organic contaminants. It is interesting to note that in surveys of organic contaminants in surface waters, ultrafiltration often is used to remove particulate matter, but it is not considered that adsorption of soluble contaminants onto the filter may alter the sample.

LOSSES DURING VACUUM DISILLATION AND LYOPHILIZATION

The original parfait method used vacuum distillation to concentrate aqueous eluates from each of the parfait beds and the aqueous effluent from the parfait column itself. The modified parfait method uses this method to concentrate only the aqueous column effluent.

To explore the potential for loss of moderately volatile compounds during vacuum distillation, solutions of biphenyl (4 μg/ml), phenanthrene (4 μg/ml) and pyrene (0.14 μg/ml) were freeze dried or vacuum distilled for varying times, and the amount of compound remaining

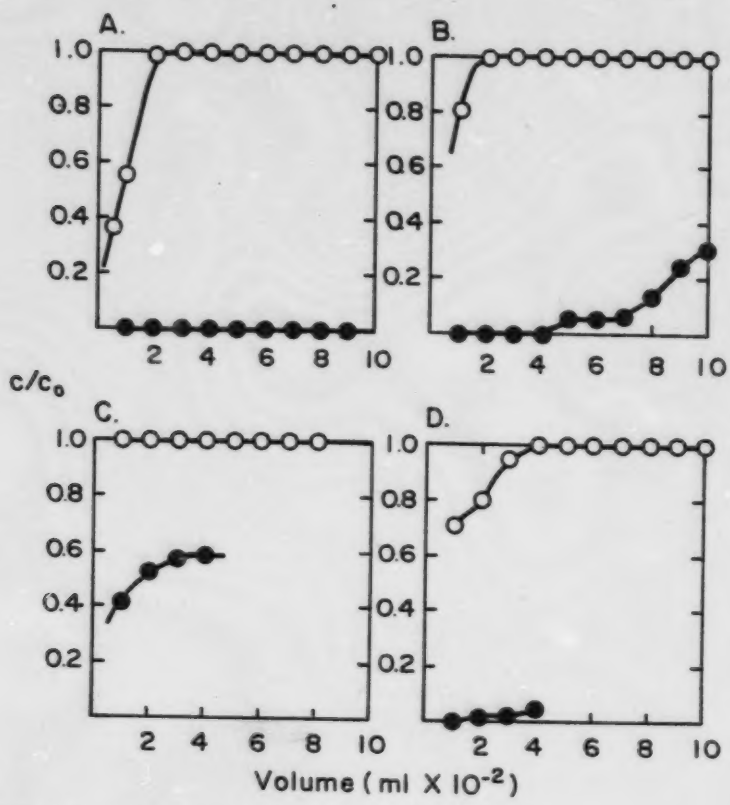

Figure 3. Adsorption of ethidium bromide and pyrene onto ultrafilters. Aqueous solutions of ethidium bromine (o) or pyrene (•) were passed through (A) Millex 0.22-µm disposable filter units; (B) Amicon sterilet disposable filter units; and Nuclepore 0.22-µm micron polycarbonate membranes in a reuseable polycarbonate holder with (D) or without (C) a fiberglass prefilter.

in the flask was determined. The results (Table 3) show that vacuum distillation resulted in greater losses than did lyophilization, and that ease of loss varied considerably. Biphenyl was so readily lost that the mere degassing of its solution resulted in a loss of about two-thirds of the original compound (see Figure 4).

To be certain that the compounds were actually escaping from the flask, rather than being adsorbed onto glass, we conducted another experiment using biphenyl (4 µg/ml), quinoline (15 µg/ml) and quinaldic acid (15 µg/ml). One hundred milliliters of each of these solutions was frozen in a 500-ml round-bottom flask and freeze dried. A trap chilled with dry ice and propanol was placed in line between the flask and the

TABLE 3. RETENTION OF BIPHENYL, PHENANTHRENE, AND PYRENE IN AQUEOUS SOLUTIONS DURING VACUUM DISTILLATION AND LYOPHILIZATION

Compound	Amount Remaining (of 5-ml sample)			
	Freeze Drying		Vacuum Distillation	
	volume (ml)	compound (%)	volume (ml)	compound (%)
Biphenyl (4 µg/ml)	4.2	87	5.0[a]	26
	2.0	47	4.9	10
	0.5	6	2.0	6
			0.5	4
Phenanthrene (4 µg/ml)	3.2	48	4.5	48
	1.0	32	1.5	19
	0.5	13	0.5	13
Pyrene (0.15 µg/ml)	5.0[a]	100	5.0[a]	100
	0.5	62	0.5	19

[a] Sample was exposed to vacuum for 60 s to degas.

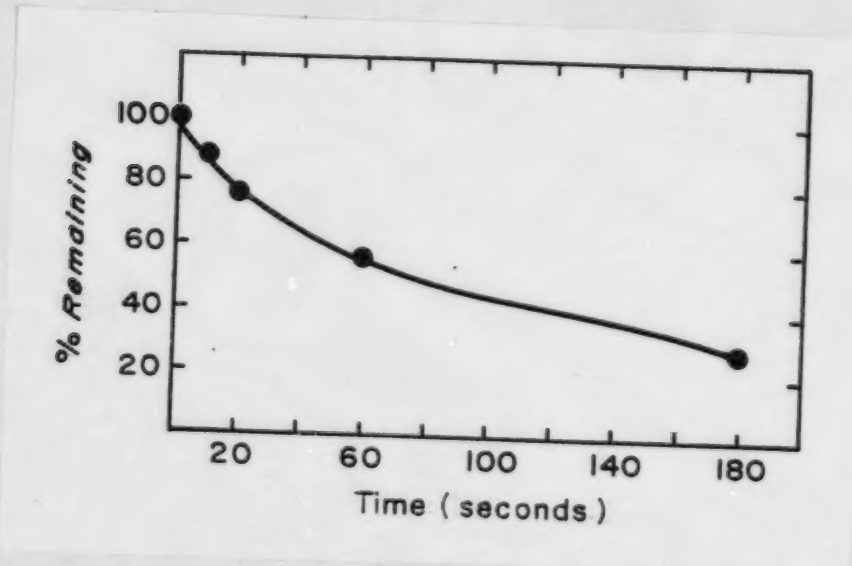

Figure 4. Loss of biphenyl during the degassing of an aqueous solution under vacuum.

mechanically refrigerated trap of the freeze drier. Compounds in the flask and the dry ice trap were quantified after approximately half of the water had sublimed from the flask. The results (see Table 4) showed that the losses of quinoline were accounted for by the material in the trap and that initially the lost biphenyl was recovered from the trap. The biphenyl was so volatile that 10 to 40% of it apparently escaped from the trap during subsequent lyophilization of half of the original solution. The quinaldate was not lost from the flask. (It will be shown in later sections that D-glucose also does not volatilize during vacuum distillation of parfait column effluents.) Thus, the losses of aromatic hydrocarbons were due to volatilization under vacuum, especially during the degassing phase of vacuum distillation. These experiments illustrate that vacuum distillation can be used only to concentrate aqueous solutions of compounds whose volatility is less than that of pyrene. Otherwise, unacceptable losses of compound may occur.

ALTERNATIVES TO SILICA GEL AS FIRST PARFAIT BED

The functions of the first parfait bed are to filter particulate matter from the sample and to adsorb hydrophobic compounds that would otherwise adhere to the polystyrene divinylbenzene backbone of the ion exchange resins. Since, in principle, a bed of polystyrene divinylbenzene resin such as XAD-2 could be added after a filtering agent, it is possible to separate the filtering function of this bed from its adsorption function. Various porous agents capable of filtering an aqueous sample were tested for their adsorptive capacities, to see if any medium combined both functions.

In a test of adsorptivity, 2-l aliquots of EB, 10 µg/l in synthetic hard water, were passed through 50-ml beds of various potential filtering agents, and extracts of the bed effluent were assayed by fluorescence. The effluent was evaporated to dryness, and the residue was redissolved in water; material remaining on the bed was extracted with acetone, evaporated to dryness, redissolved in acetone, and analyzed. EB was chosen as the first test compound because it is easily and sensitively assayed, it is essentially nonvolatile, it is water soluble, and it has both hydrophobic (aromatic) and cationic functional groups.

The results (Table 5) show several unusual features. Inert materials, including powdered cellulose, Celite, and porous Teflon, each adsorbed appreciable amounts of EB. The total recovery of EB from the filtering agent and the effluent was nonquantitative for all materials except porous Teflon. Florisil, silanized silica gel, porous Teflon, and silica gel adsorbed virtually all of the EB, as reflected by an absence of the compound in the column effluent, but the compound was not extractable with acetone from florisil or silica gel. At the top of the florisil bed, a thin red band was easily seen, indicating a very strong adsorption of the EB. Attempts to elute EB from florisil with acetone, benzene, methanol, 15% NaCl, 2 M triethylammonium bicarbonate pH 7.5

TABLE 4. LOSS OF BIPHENYL, QUINOLINE, AND QUINALDIC ACID FROM 100 ml OF AQUEOUS SOLUTION DURING LYOPHILIZATION

	Retained in Flask		Recovered in Trap(%)
	volume (ml)	compound (%)	
Biphenyl (4 µg/ml)	55, 53 100[a]	45, 31 45	44, 29 54
Quinoline (15 µg/ml)	52	19	80
Quinaldic acid (15 µg/ml)	50, 52	100, 100	not detected

TABLE 5. RETENTION OF ETHIDIUM BROMIDE ON FILTERING AGENTS[a]

	EB Recovered (µg)			
Filtering Agent	in effluent	from filtering agent	total	Percent EB Recovered
Silica gel	0	0	0	0
Florisil	0	0	0	0
Cellulose powder	4	8	12	57
Celite	4	13	17	85
Glass wool	1	12	13	65
Silanized silica gel	<1	8	8	37
Chromosorb T	<1	19	19	95

[a] In each case, 2 l of EB (10 µg/l) in synthetic hard water was passed over a 50-ml water-washed bed of the filtering agent. The agent was eluted with acetone, the effluent was vacuum distilled to dryness, and the EB was recovered in water.

($TEAHCO_3$), 0.10 M EDTA, and 1 M NaOH were unsuccessful. Only the NaOH moved the red band from the top of the bed, suggesting that solubilization of the florisil was necessary to liberate the compound.

The failure to recover EB in the acetone extract from the silica gel is also striking. In early experiments, elution of EB adsorbed to silica gel was attempted first with $TEAHCO_3$ pH 7.5 and then with acetone, and as much as 22% of the EB was recovered. The difference between this result and the failure to elute EB in acetone alone could be explained if the ethidium ion were bound to silica by ion exchange. Acetone alone provides no mobile counterion; therefore, the ethidium ion could not be recovered in it. However, if mobile counterions were supplied by a residue of $TEAHCO_3$ from a first aqueous elution step, then some ethidium bicarbonate apparently could dissolve in the subsequent acetone eluant. This explanation is also supported by recovery of 37% of the EB applied to the silanized silica gel, a medium in which acidic (ion exchange) moieties on the silica are blocked (see Table 5). Failure to quantitatively recover EB from either kind of silica gel, however, indicates that adsorption of this compound was not exclusively an ion exchange process.

These exploratory studies with EB led to three further lines of testing. The results for EB elution from silica gel by ionic versus neutral organic eluants suggested experiments on selective elution of organic acids and bases from ion exchange resins, described in a later section. The results with silanized silica gel suggested that even silanized glass surfaces might exhibit an affinity for hydrophobic aromatic compounds. This idea is explored in the next section. Finally, the results suggested that porous Teflon might be a desirable first adsorbent for the parfait system, because it would serve as an inert filtering medium but it would also reversibly bind certain trace organic contaminants in water. Teflon was the only medium tested that exhibited this reversible binding.

Adsorption Onto Silanized Glass

The original parfait column was composed entirely of glass, fiberglass, and Teflon. As was shown in the section on the adsorptivity of ultrafilters, fiberglass prefilters such as those originally used to support the parfait beds can adsorb appreciable amounts of aromatic hydrocarbons. Silanization was expected to reduce this affinity, but the result with silanized silica gel suggested that even silanized glass surfaces might remain strongly adsorptive. To test the suitability of silanized glass surfaces for use in a modified parfait system, we determined the breakthrough of caffeine (15 mg/l), pyrene (0.14 mg/l), and EB (10 mg/l) from 5-ml beds of silanized glass wool and silanized 60–80 mesh glass beads (see Figure 5). Only caffeine showed no appreciable adsorption. Both pyrene and EB were removed essentially quantitatively from the first two column volumes of solution, and pyrene in the effluent did not reach 50% of the influent concentration until

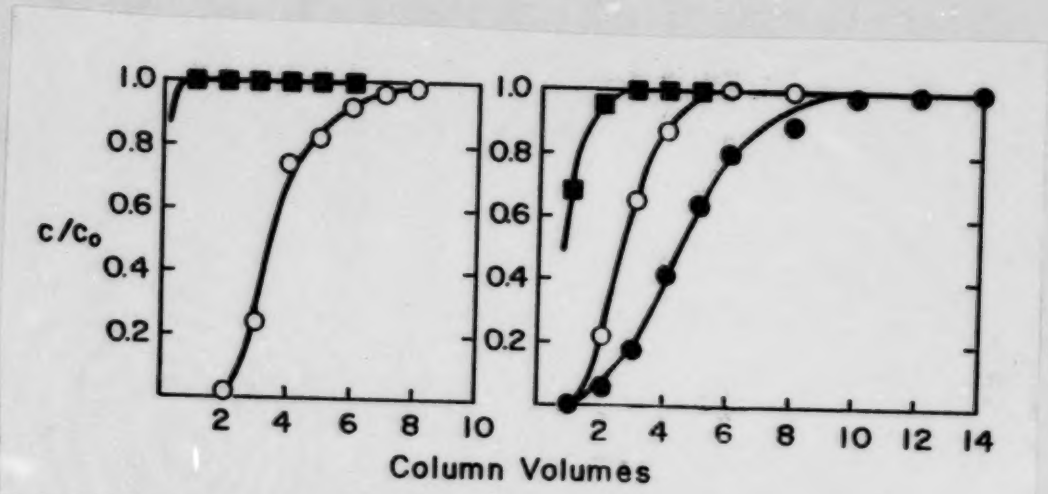

Figure 5. Adsorption of solutes to silanized glass wool and silanized glass beads. Aqueous solutions of caffeine (■), 15 mg/1; ethidium bromide (o), 10 mg/1; or pyrene (●), 0.14 mg/1 were passed through 5-ml beds of silanized glass wool (left panel) or silanized glass beads (right panel).

five column volumes had been collected. Despite the adsorptivity of these media, the adsorbed compounds could be recovered in acetone washings. Thus, silanized glass and fiberglass were considered acceptable for use in the modified parfait method.

Characterization of Porous Teflon

The preliminary work with EB had suggested not only that Chromosorb T might be an effective agent for filtering particulate matter from water, but that it might reversibly adsorb compounds of aromatic character. We explored the recovery of some of the model compounds to determine whether a detailed characterization of the properties of porous Teflon was warranted. Solutions of pyrene (0.14 mg/1), EB (10 mg/1), glucose (50 mg/1), caffeine (15 mg/1), quinaldic acid (15 mg/1), glycine (10 mg/1), and benzylamine (15 mg/1) were passed through 3-ml beds of Chromosorb T in 1.2 cm i.d. glass columns, and the effluent was monitored for compound by absorbance, fluorescence, fluorescamine assay (glycine), or glucose oxidase/o-dianisidine assay (glucose). Glucose, quinaldic acid, glycine, and benzylamine did not show detectable adsorption to the Teflon. In contrast, pyrene was so strongly adsorbed that none broke through in up to 130 bed volumes, the maximum tested in this experiment. Similarly, EB did not break through in up to 40 bed volumes, the maximum volume tested. Caffeine began to appear in the effluent after 10 bed volumes had passed through, and its effluent concentration reached 50% of the influent concentration at 16.0 bed volumes. It therefore appeared that Chromosorb T adsorbed the larger aromatic compounds but showed essentially no affinity for the neutral, water-soluble species, even when these had up to one aromatic nucleus.

It is essential that compounds adsorbed can be recovered from the Teflon, if it is to be useful for the parfait system. EB was recovered essentially quantitatively in the first experiments. An 8-l mixture of 2,2′-dichlorobiphenyl, anthraquinone, stearic acid, BHT, and bis(2-ethylhexyl)phthalate, each 50 µg/l in synthetic hard water, was passed through a 50-ml bed of Chromosorb T, and the bed eluted with methylene chloride (CH_2Cl_2). The recovery of each compound was determined by gas chromatography after concentration and methylation as indicated in Section 4, Materials and Methods. Table 6 presents the recoveries. At least half of each compound was adsorbed by the Teflon and recovered. The data for the three trials are recorded in the order the trials were done, suggesting that technique and recovery improved with experience. (In later experiments, quantitative recoveries of each of these compounds except dichlorobiphenyl were achieved when the compounds were tested in solutions containing humic acid.) Based on these results, we proceeded with a detailed analysis of the adsorptive properties of porous Teflon, to evaluate it for use as the first bed in the modified parfait system.

Characterization of Adsorption by Porous Polymers

The adsorption of compounds onto polymers can be characterized using static equilibria (Burns and Smith, 1980) or migration of solutes on columns (Thurman et al., 1978; Jahangir and Samuelson, 1980; Bacaloni et al., 1980; Lagana et al., 1980; Leoni et al., 1976; Navratil, 1977). In column studies, frontal chromatography is used to determine the capacity of the adsorbent (k′) from the breakthrough curves. In some experimental systems, however, breakthrough curves deviate strongly from ideal shapes. For this reason, among others, capacities have been reported in terms other than k′. Examples include capacities reported as the total amount of solute adsorbed before the effluent concentration reached 1 ppm (Kawabata and Ohira, 1979); the retention volume when the effluent concentration equalled 50% of the influent concentration (Leenheer, 1981); the mass of solute adsorbed divided by the mass of solute in the void volume, determined by elution (Thurman et al., 1978); the effluent volume at the moment when the effluent concentration equalled 1% of the influent concentration (Jahangir and Samuelson, 1980); and the effluent volume when the effluent concentration equalled 10% of the influent concentration (Picer and Picer, 1980).

TABLE 6. RECOVERY OF SOLUTES FROM CHROMOSORB T IN CH_2Cl_2

Solute	Percent Recovery	Mean ± S.D.
2,4′-Dichlorobiphenyl	83, 76, 89	83 ± 6
Anthraquinone	43, 53, 95	64 ± 27
Stearic acid	56, 53, 85	65 ± 18
Bis(2-ethylhexyl)phthalate	50, 62, 75	62 ± 13
BHT	50, 74, 77	67 ± 15

Breakthrough curves are usually presented as the ratio of effluent concentration, c, to influent concentration, c_o, versus the volume of effluent. A typical breakthrough curve on porous Teflon is shown in Figure 6. In general, breakthrough curves on porous PTFE columns appear linear between $c/c_o = 0.1$ and $c/c_o = 0.6$, and they closely approach ideal shape.

By the fundamental equation of liquid chromatography,

$$V_R = V_o (1 + k'),$$

the capacity factor, k', can be related to the void volume, V_o, and the retention volume V_R (Kucera et al., 1981). For this report, V_R is defined as the experimental effluent volume when $c/c_o = 0.5$. We estimated the breakthrough volume, V_B, as the x-intercept of the line tangent to the breakthrough curve between $c/c_o = 0.1$ to 0.6. If the profile of the front conforms to plate theory, the frontal step width, w (see Figure 6) can be expressed as units of volume, $w = 2(V_R - V_B)$, and related to the number of theoretical plates, N, by the equation

$$N = 2(V_R/w)^2.$$

Thurman et al. (1978) determined capacity factors for 20 related organic solutes adsorbed from water on XAD-8. They showed a linear relationship between the logarithm of the capacity factor and the logarithm of the aqueous molar solubility of these solutes. We used breakthrough curves to determine capacity factors and then examined this relationship between capacity factor and solubility for solutes adsorbed by Chromosorb T.

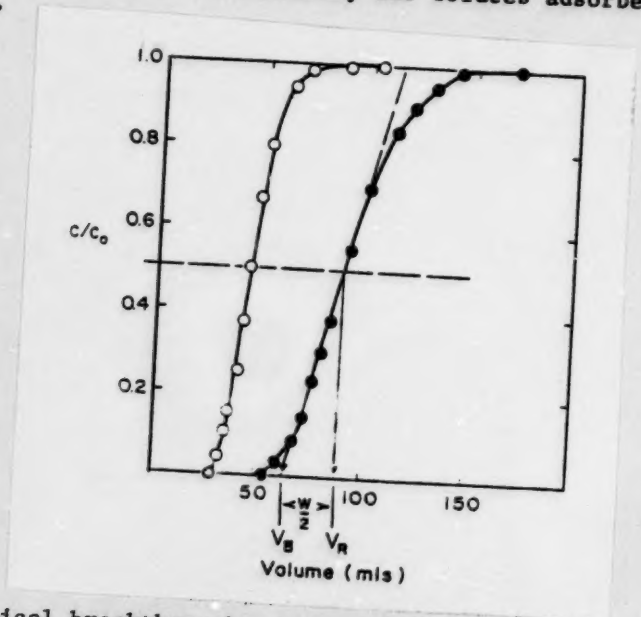

Figure 6. Typical breakthrough curves for solutes on porous Teflon. The breakthrough of caffeine (o) and quinoline (•) from a 5-ml bed is shown, and the relationship of V_B, V_R, and w is illustrated.

CHARACTERIZATION OF POROUS TEFLON

To determine k′ from a breakthrough curve, the void volume, V_o, must be known. This was determined by two methods on two kinds of Teflon particles, Chromosorb T and Fluoropak 80. Both Teflons contain similar-sized particles that are actually aggregates of even smaller particles. The manufacturers stated that the total surface area per unit weight of these two kinds of Teflon particles differed by a factor of about two (see Section 4, Materials and Methods). By comparing these two Teflons, we hoped to demonstrate that adsorptive capacity depended on available surface area.

The void volume of porous Teflon packings is dependent upon the displacement of air from the internal spaces of the aggregate by the packing solvent. As shown in Table 7, dry-packed columns have almost no void volume, since water by itself is unable to displace air from cavities in the Teflon aggregate. PTFE surfaces have an extremely low surface free energy and are only fully wetted by solvents with surface tensions below 20 dyne/cm (Bikales, 1971). In principle, water-miscible solvents having low surface tension are able to displace air from the Teflon surface and then be replaced by water. Experiments have shown, however, that effective packing solvents include protic and aprotic, high dielectric compounds such as methanol, acetone, and p-dioxane. At room temperature these have surface tensions of 22, 23, and 33 dyne/cm, respectively. All of these exceed the maximum surface tension for complete wetting of PTFE surfaces. Acetonitrile, a water-miscible

TABLE 7. VOID VOLUMES OF POROUS TEFLON COLUMNS[a]

Polymer	Packing Solvent	V_o (ml) by: ^3H_2O (n)	Weight (n)
Chromosorb T	none (dry)		1.11 ± 0.33 (3)
	acetone	4.07 ± 0.01 (3)	4.17 ± 0.44 (6)
	acetonitrile	2.53 ± 0.14 (3)	2.75 ± 0.10 (3)
	methanol		4.06 ± 0.20 (3)
	tetrahydrofuran		4.24 ± 0.12 (3)
	p-dioxane		4.12 ± 0.03 (3)
Fluoropak 80	none (dry)		0.96 ± 0.09 (3)
	acetone	3.23 ± 0.14 (3)	3.45 ± 0.12 (3)
	acetonitrile	2.32 ± 0.06 (3)	2.51 ± 0.19 (3)

[a] Each column contained 2.65 g of Chromosorb T or 3.70 g of Fluoropak 80, giving a 5-ml packed bed volume. Void volumes were determined as described in Section 4; the mean, standard deviation, and number of determination are presented.

high dielectric, aprotic solvent, has a surface tension of 29.1 dyne/cm at 20°C, (Thon, 1941) but it is unable to displace most of the air from the internal spaces of the Teflon aggregates. This is reflected not only by the lower V_o of acetonitrile-packed columns, Table 7, but by the noticeably greater opacity of acetonitrile suspensions of PTFE compared to the greyer, translucent appearance of PTFE suspensions in the other solvents. Also, when equal weights of porous Teflon are suspended in equal volumes of acetone or acetonitrile in a graduated cylinder, the volume of the acetonitrile suspension is greater than the acetone suspension by an amount per weight of polymer that corresponds to the differences in V_o of acetone- or acetonitrile-packed columns shown in Table 7.

The appearance of platinum-shadowed Chromosorb T and Fluoropak 80 in the scanning electron microscope shows a physical basis for these observations, Figure 7. Both polymers were manufactured from a colloidial dispersion of PTFE particles called Teflon-6 (unpublished manufacturers product information) by sintering near to their melting temperature. The details of the manufacture are not available, but from the appearance of the aggregates in Figure 7b, it is apparent that Chromosorb T retains much of the structure of the starting colloid, appearing as a discernable aggregate of 0.2 μm diameter globules. Most of the globules in Fluoropak 80, in contrast, have fused to give a continuous macro-structure; only at highest magnification is the original globule-like starting material revealed by the texture of the surface and by the size of the holes in it. In the Chromosorb T aggregate, the spaces between adjacent globules on the surface are 20 to 40 nm. However it is not known whether the dimension of the spaces on the surface are typical of those in the interior of the aggregate.

In principle, the capacity of a porous Teflon for a particular compound should be a function of total surface area. The manufacturers' stated range of surface area is 2-4 m^2/g for Fluoropak 80 and 4-7 m^2/g for Chromosorb T. These ranges were determined by the Brunauer, Emmitt and Teller gas adsorption method (personal communication, R.J. Zilli, Manville Products Corp.). The morphology of the two PTFE aggregates can account for these differences in surface area per unit weight. We next explored the possibility that surface area limits adsorptive capacity.

Table 8 presents data on the capacity of Fluoropak 80 and Chromosorb T for various solutes, while Table 9 compares the capacity of acetonitrile-packed Chromosorb T to polymer packed in acetone or methanol. There is a striking difference in the capacity of Chromosorb T and Fluoropak 80. On the basis of the average total surface area of a 5-ml column of the two polymers, Chromosorb T should show only 1.3 times the capacity of Fluoropak 80 for any solute. The observed ratios of the capacity of Chromosorb T to Fluoropak 80, for 3 solutes, in terms of nmoles of solute adsorbed, was 6 to 14, Table 8. Moreover, when the internal surfaces of Chromosorb T were blocked with air by packing in acetonitrile (Table 9), its capacity for acenaphthene decreased five-fold.

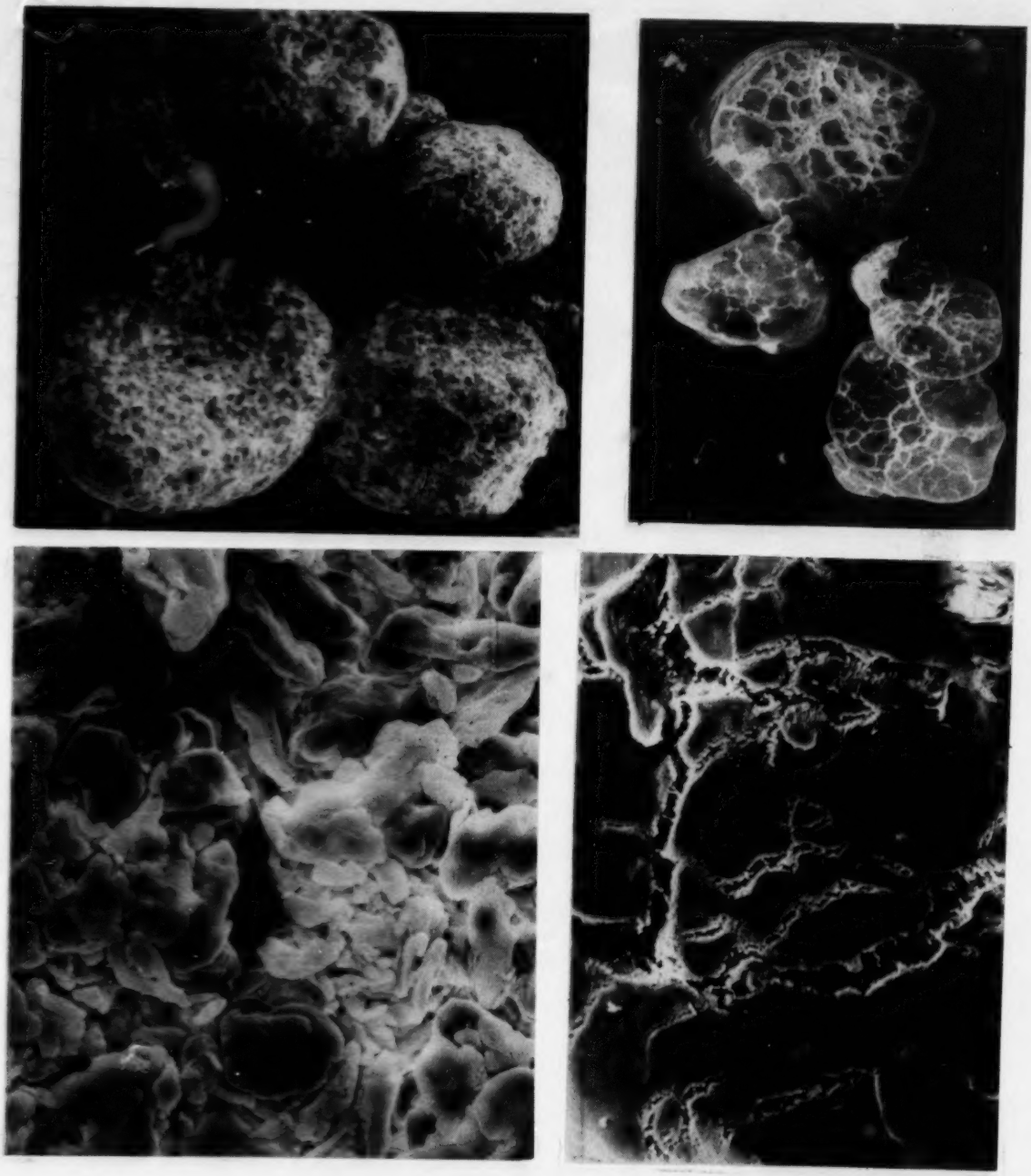

Figure 7a. Scanning electron micrographs of platnium-shadowed Fluoropak 80 (left) and Chromosorb T (right). The top panels are magnified 60x, the bottom panels 1000x.

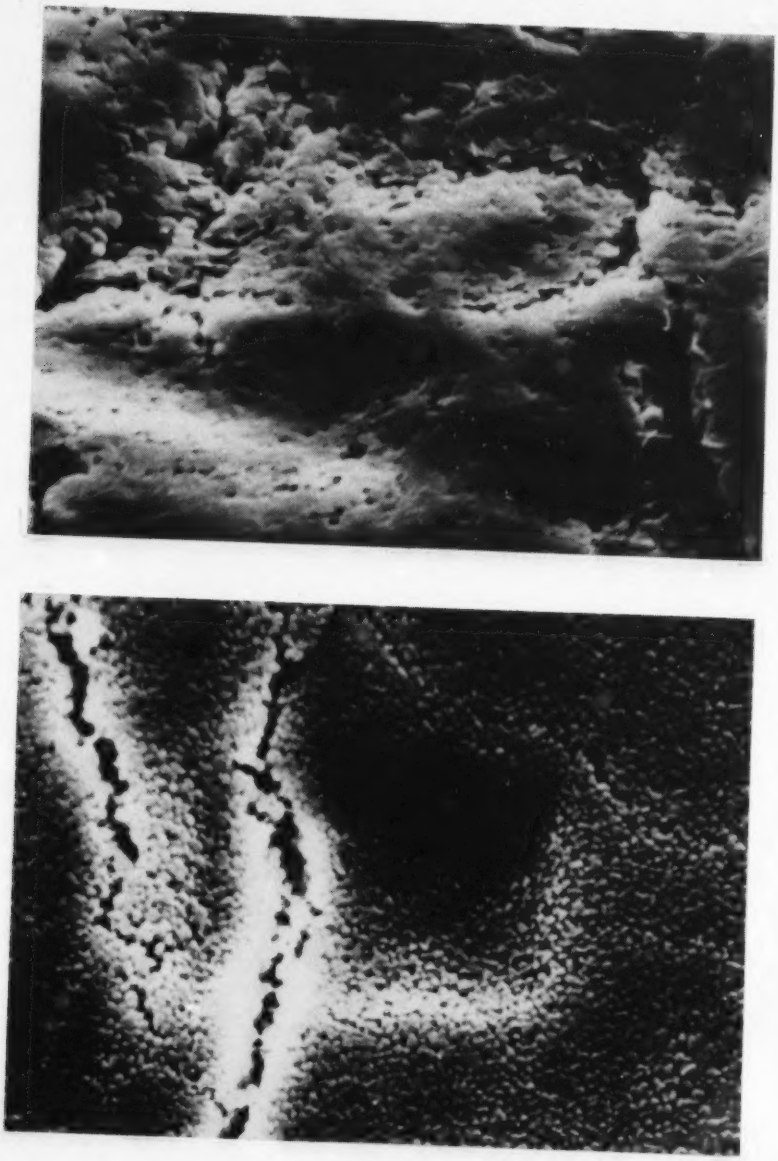

Figure 7b. Scanning electron micrographs of Fluoropak 80 (top) and Chromosorb T (bottom) magnified 6,600x.

TABLE 8. CAPACITIES OF CHROMOSORB T AND FLUOROPAK 80[a]

Solute	Adsorbent	V_B (ml)	V_R (ml)	k'	n
Caffeine	Fluoropak 80	5	7	1.2	1
	Chromosorb T	59 ± 5	95 ± 11	22 ± 3	4
Theophylline	Fluoropak 80	< 5	< 5	< 1	1
	Chromosorb T	23	39	8.6	1
Methyl-3-isobutyl-xanthine	Fluoropak 80	16	32	9.0	1
	Chromosorb T	122	179	43	1

[a] Each column contained 2.65 g of Chromosorb T or 3.70 g of Fluoropak 80 packed in acetone and washed before use with 100 bed volumes of synthetic hard water.

TABLE 9. INFLUENCE OF PACKING SOLVENT ON CAPACITY OF CHROMOSORB T FOR ACENAPHTHENE

Packing Solvent	Concentration (mg/l)	V_B (ml)	V_R (ml)	k'	n
Acetone	2.5	87 ± 7	174 ± 7	41 ± 2	3
	0.1	96 ± 25	231 ± 35	56 ± 9	3
Acetonitrile	2.5	1 ± 2	42 ± 8	9 ± 2	3
	0.1	10	45 ± 9	10 ± 2	3
Methanol	2.5	82	190	46	1
	0.1	90	190	46	1

The differences in the adsorptive capacity of Chromosorb T and Fluoropak 80 cannot be accounted for on the basis of surface area alone. The results in Table 8 fall outside the range of possible capacity ratios even if Fluoropak 80 had the minimum area (2 m^2/g) and Chromosorb T had the maximum area (7 m^2/g) reported by the manufacturer. Under the conditions above, and assuming equal adsorptivity per unit surface area, the ratio of the capacity of Chromosorb T to Fluoropak 80 should be 2.5 (the observed ratios were 6 to 14). If adsorptivity were a simple proportion of total surface area, the Chromosorb T would have to have an area of 17 to 72 m^2/g, depending on whether the Fluoropak 80 were 2 or 4 m^2/g, to account for the observed capacity ratios.

The observed ratios of the capacity of Chromosorb T to that of Fluoropak 80 could be accounted for in several ways. It is possible that the manufacturers' stated surface area underestimates the true surface area of Chromosorb T. Figure 7b revealed that Chromosorb T was made up of globules approximately 0.2 μm in diameter. If Chromosorb T was composed of solid Teflon spheres of this diameter, and having a density of 2.10 g/ml (Bikales, 1970), a surface area in excess of 1000 m^2/g would be possible. The results could also be explained if the interior surface of Chromosorb T had a higher adsorptive capacity than the exterior surfaces. The void volumes of Fluoropak 80 and Chromosorb T in acetone and acetonitrile (Table 7) and the electron micrographs (Figure 7) show that Chromosorb T has a higher proportion of internal surfaces than Fluoropak 80. The reduction of capacity when Chromosorb T was packed in acetonitrile (Table 8) also suggests that the majority of the adsorptive capacity is associated with internal surfaces.

At least two plausible hypotheses could account for a dependence of adsorptivity upon internal surfaces. The first hypothesis is that the internal space harbors residual solvent that exists as an adsorptive phase separate from the Teflon or the water phases. Such a phase might be a microlayer of solvent stabilized by the proximity of two Teflon surfaces in the internal space, but not stable when in association with only one Teflon surface on the exterior of the particle. Alternatively, residual solvent might actually exist in pockets stabilized in some way by the internal space. In either case, the distances between particles seen in Figure 7b are enough to accomodate solvent layers at least hundreds of molecules thick.

The second hypothesis assumes that no residual solvent remains in the internal space and that the Teflon surfaces in close proximity modify the structure of water in some way, giving it solvent-like properties. A similar interpretation was given by Yano and Janado (1980) to explain the adsorptivity of certain dextran polymers. In the case of Teflon, it may be the intense electronegativity of the two opposing surfaces that enables them to order the water structure, through electrostatic, hydrogen-bonding, or other influences.

A basic difference between these hypothesized mechanisms would be the stability of the adsorptive capacity. If residual solvent actually

resided in the internal Teflon spaces, then it would exchange with water over time, causing capacity to decrease. Also, packing the Teflon column in different solvents would presumably produce different adsorptive capacities, a prediction not supported by the data given in Table 9, where methanol- and acetone-packed Chromosorb T columns showed identical capacities for acenaphthene. Similarly, only little solvent should remain on a Teflon surface, because of the high water miscibility of acetone and methanol.

If solvent in the internal space were responsible for the adsorption, then the quantitative adsorption of a compound on a Teflon column might be readily saturable, resulting in lower capacity factors in response to higher solute concentrations. Also, faster flow rates might give less time for equilibration between phases and thereby decrease capacity. The results shown in Table 10 demonstrate no decrease in capacity of acetone-packed Chromosorb T when the concentration of acenaphthene or quinoline was varied twentyfold. Also, flow rate had only a small effect. Such experiments are not conclusive, however, because it is always possible that the particular solute and experimental conditions tested were not sufficient to discernably limit the partition of solute into the solvent-medium. Even experiments in which acetone-packed Teflon was washed with 5, 100, or 400 column volumes of water and showed no discernable change in capacity fail to disprove the associated-solvent hypothesis, because it can always be postulated that the association of the solvent with the Teflon surface is too strong to be affected by washing with these volumes of water. However, the results of these experiments and those in Tables 9 and 10 are more simply explained by the alternative hypothesis that the water contained in the internal Teflon spaces is modified to give it solvent-like qualities.

A prediction of the modified-water hypothesis is that water-structure breakers like urea will decrease the capacity of Teflon for hydrophobic solutes. This prediction is supported by the data in Table 11, which show that urea severely decreased Chromosorb T's capacity for acenaphthene. Other interpretations of this experiment invoking interactions between urea and solute or urea and Teflon are also possible, however.

For the present we conclude that columns of porous Teflon (i.e., Chromosorb T) have a void volume that includes an internal and an exterior volume. Also, it is evident that the internal volume must be wetted with solvent if the high adsorptive capacity of the polymer is to be exhibited. The hypothesis that water contained in the internal volume is modified by the Teflon to give it solvent-like properties is the simplest explanation of the data now available.

TABLE 10. INFLUENCE OF CONCENTRATION AND FLOW RATE ON BREAKTHROUGH FROM CHROMSORB T

Solute	flow rate (ml/min)	Solute concentration (mg/l)	V_B	V_R	n
Acenaphthene	1.7	2.5	85 ± 6	200 ± 27	4
	1.7	0.62	92 ± 3	223 ± 6	3
	1.7	0.12	95	205	1
	1.7	0.10	100 ± 14	215 ± 39	5
Quinoline	1.7	0.1	25	42	1
	1.7	15	27 ± 5	40 ± 3	3
	5.5	15	22	36	1

TABLE 11. INFLUENCE OF UREA ON THE CAPACITY OF CHROMOSORB T FOR ACENAPHTHENE[a]

Solvent	V_B	V_R	k'	n
Synthetic Hard Water (SHW)	92 ± 3	223 ± 6	54 ± 2	3
SHW + 1 M	70	150	36	1
SHW + 2 M	40	105	25	1
SHW + 4 M	35	78	18	1
SHW + 6 M	20	44	10	2

[a] Acenaphthene concentration was 0.62 mg/1 in each case.

Capacity Of Chromosorb T For Aromatic Compounds

Regardless of the mechanism(s) of the adsorption, Chromosorb T can be characterized as an adsorbent useful in the parfait method. The capacities of Teflon for the compounds listed in Table 12 were determined from breakthrough curves, using 5-ml acetone-packed beds of Chromosorb T washed before use with 100 bed volumes of synthetic hard water. In each experiment, flow was restricted to 1.7 ml/min. Fractions (4.7 ml) were collected and the concentrations of solutes in the effluent determined as described in Section 4, Materials and Methods.

Compounds that showed breakthrough in less than one column volume were glucose, glycine, tryptophan, adenine, uracil, xanthine, trimesic acid, benzylamine, and the anionic dye rose bengal. Compounds that did not break through in up to 1400 bed volumes, the maximum volume tested, were 2,2′-biquinoline and the cationic dyes crystal violet, safranin O and ethidium bromide. The latter three compounds are readily soluble in water, unlike the other compounds that adsorb to Teflon.

The first seven compounds in Table 12 are aromatic hydrocarbons that constitute a series of functionally similar solutes. According to Locke (1974) and Thurman et al. (1978), the relative retention of functionally similar solutes on hydrophobic adsorbents is determined by their relative solubilities in the mobile phase. This adequately describes the behavior of these solutes on Chromosorb T (Tables 12 and 13 and Figure 8).

MacKay et al. (1980) discussed the thermodynamic relationship between aqueous solubility and octanol-water partition coefficient K_{ow}, suggesting that correlations of the partitioning of solutes from water onto sorbents should be made with the octanol-water partition coefficient rather than with molar aqueous solubility, since the partition coefficient is a more direct measure than solubility of the primary parameter governing the adsorption, the aqueous activity coefficient of the solute. The results shown in Figure 9 are consistent with this expectation, showing a slightly better correlation of capacity with octanol-water partition coefficient ($r^2 = 0.96$) than with solubility ($r^2 = 0.94$) (Figure 8).

Also shown on Figure 8 are values for the capacity factors of various nitrogen-containing heterocyclic aromatic compounds. These do not fit the correlation described by the hydrocarbons; this is not surprising, because the heterocycles are not functionally related to the hydrocarbons. Among the nitrogen heterocyclics, the methyl-substituted xanthines show a tendency for capacity on Tefon to be correlated with solubility. This is highly unexpected because all previously observed correlations of capacities are with the reciprocal of solubility, not directly with solubility. The roughness of the correlation may be due in part to the imprecision in the values for the solubility of these compounds. Nevertheless, the trend seems clear.

TABLE 12. BREAKTHROUGH OF VARIOUS ORGANIC COMPOUNDS FROM 5-ML COLUMNS OF CHROMSORB T

Solute	Concentration (mg/l)	V_B (ml)	V_R (ml)	k′
Fluoranthene	0.1	3300	6000	1470
9-Methylanthracene	0.1	2500	6000	1470
Pyrene	0.1	2300	4600	1130
Phenanthrene	0.1	540	1500	368
Fluorene	0.1	400	1100	269
Acenaphthene	0.1	96	231	54
Biphenyl	15	92[a]	230[a]	93[a]
1-Naphthol	0.1	25	38	8.3
2,4-Dichlorophenol	15	10	27	5.6
Acridine	15	660	1100	269
Quinoline	15	27	40	8.8
Indole	15	7	12	1.9
3-Methylxanthine	15	6	10	1.4
3,7-Dimethylxanthine (theobromine)	15	18	28	5.8
1,3-Dimethylxanthine (theophylline)	15	23	39	8.6
1,3-Dimethyl-7(2,3-dihydroxypropyl)xanthine (7(2,3-dihydroxypropyl)theophylline)	15	34	53	12
1,3,7-Trimethylxanthine (caffeine)	15	59	94	22
1,3-Dimethyl-7(2-chloroethyl)xanthine (7(2-chloroethyl)theophylline)	15	70	100	24
1-Methyl-3-isobutylxanthine	15	122	179	43

[a] Determined on a 3.0-ml column.

TABLE 13. CAPACITY ON TEFLON, SOLUBILITY, AND OCTANOL-WATER PARTITION COEFFICIENT OF VARIOUS SOLUTES

	log k′	log (nmol adsorbed)	log (solubility)[a] (μ mol/l)	log K_{ow} [a]
Fluoranthene	3.17	3.47	0.11	5.22
9-Methylanthracene	3.17	3.48	0.12	5.14
Pyrene	3.05	3.36	-0.18	5.12
Phenanthrene	2.57	2.92	0.86	4.46
Fluorene	2.43	2.82	1.08	4.18
Acenaphthene	1.73	2.18	1.41	4.03
Biphenyl	1.97[b]	-	1.66	4.09
1-Naphthol	0.92	1.42	3.7	2.98
2,4-Dichlorophenol	0.75	3.40	4.44	-
Acridine	2.43	4.96	2.33	3.6
Quinoline	0.95	3.66	5.7	2.03
Indole	0.29	3.19	4.48	2.14
3-Methylxanthine	0.16	2.96	-	-
Theobromine	0.77	3.37	3.4	-0.78
Theophylline	0.93	3.51	4.7	-0.02
7(2,3-Dihydroxypropyl)theophylline	1.08	3.52	6.1	-
Caffeine	1.34	3.86	5.0	0.01
7(2-Chloroethyl)theophylline	1.37	3.82	-	-
1-Methyl-3-isobutylxanthine	1.63	4.08	-	-

[a] Data from MacKay et al., 1980; Hansch and Leo, 1979.
[b] Determined on a 3-ml column.

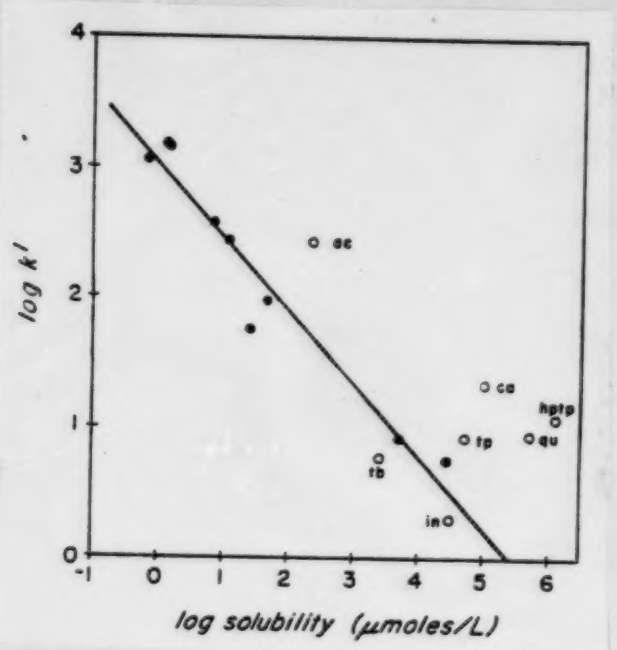

Figure 8. Correlation of capacity factor with solubility (μ mols/l). A least-squares fit of the data for the hydrocarbons (•) is described by log k' = -0.56 log solubility + 3.01. Nitrogen heterocycles (o) are: ac = acridine, ca = caffeine, in = indole, hptp = 7(2,3-dihydroxypropyl)theophylline, qu = quinoline, tb = theobromine, tp = theophylline.

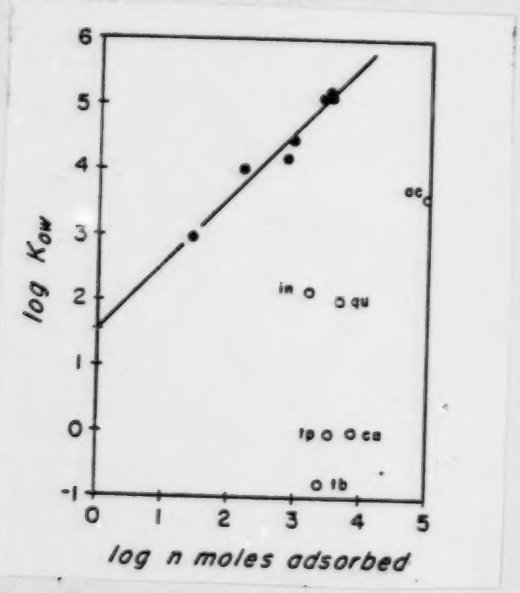

Figure 9. Correlation of capacity of a 5-ml bed with octanol-water partition coefficient. A least-squares fit of the data (•) for the hydrocarbons is described by log nmoles adsorbed = 1.03 log K + 1.55. Nitrogen heterocycles are designated as in Figure 8.

Suspecting that this relative order of capacities for the xanthines may be found only on Teflon, the capacities of XAD-2 and XAD-8 for three of these compounds were determined, Table 14. The same unexpected tendency for capacity and solubility to correlate was found using these adsorbents. The generality of the widely accepted inverse correlation of solubility and capacity factor, or direct correlation of octanol-water partition and capacity, should therefore be questioned. It should be remembered that the correlation is expected to be true only for solutes functionally related to the aliphatic, aromatic and alicyclic hydrocarbons previously used to demonstrate it (Thurman et al., 1978; Locke, 1974).

The anomalous behavior of the substituted xanthines may be due to the formation of complexes in aqueous solution. Caffeine self-associates to form dimers and tetramers in water (Guttman and Higuchi, 1957; Cesaro et al., 1980). increasing its water solubility. If oligomer formation is a general property of the xanthine series, it might account for the relationship between solubility and adsorption to hydrophobic resins. Xanthine complexes may adsorb to Teflon or to polystyrene more readily than the corresponding monomers. The complexes would thus be responsible for a higher adsorptivity and a higher water solubility than would be expected from the monomer alone.

The other anomalous adsorption discovered here, the tight association of cationic aromatic dyes with PTFE, is less straightforward. The combination of a hydrophobic and a cationic moiety on these water-soluble compounds suggests a dual interaction of these

TABLE 14. COMPARISON OF CHROMOSORB T, XAD-2, AND XAD-8 CAPACITY FOR XANTHINE COMPOUNDS

Adsorbent	Solute	V_B	V_R	k'
Chromosorb T	theobromine	18	28	6
	7(2,3-dihydroxypropyl)theophylline	34	53	12
	caffeine	59	94	22
XAD-8	theobromine	1	16	4
	7(2,3-dihydroxypropyl)theophylline	5	32	9
	caffeine	11	72	22
XAD-2	theobromine	5	100	30
	7(2,3-dihydroxypropyl)theophylline	7	152	47
	caffeine	127	446	138

solutes with the hydrophobic and strongly electronegative surface of Teflon. On the other hand trimesic acid and the anionic, aromatic dye rose bengal failed to display any affinity for Teflon, suggesting that electrostatic repulsion overcomes any affinity due solely to the hydrophobic effect. The affinity of Teflon for cationic, aromatic compounds suggests that pesticides like diquat and paraquat may also be strongly adsorbed on PTFE columns.

The characterization of Chromosorb T demonstrated that this material is an adsorbing agent comparable to the XAD resins. Per unit surface area, porous Teflon actually has a greater adsorptive capacity than the XAD's. In practice, however, the XAD's have a much higher surface area than Teflon per unit weight or per bed volume. Nevertheless, approximately four bed volumes of the Teflon will equal the capacity of one bed volume of an XAD-2 column; XAD-8 and Teflon have roughly equal capacities per bed volume. Other attributes of Teflon suggesting that it is superior to the XAD's are its chemical stability, inertness, and purity (Gangal, 1980). Finally, as described in a later section, porous Teflon has a high capacity for humic acid, at least for the sample provided for this study by HERL. In this attribute, Teflon differs from the XAD's.

Further development of the parfait method employed Chromosorb T as the first parfait bed.

ION EXCHANGE RESINS

A variety of ion exchange resins (listed in Table 15) were considered for use in the parfait column. The resins eventually chosen were selected because they carried strongly acidic or basic exchange groups, they were macroporous, they had relatively higher exchange capacity than otherwise equivalent alternatives, and they were lower in cost. MSC-1 was finally chosen over AG MP-50 primarily on the basis of cost, and A-162 was preferred over other macroporous anion exchange resins primarily because of its higher exchange capacity. A-162 gave the impression of cleaner eluates than did AG MP-1, but this was not tested rigorously in side-by-side trials. Satisfactory parfait columns could probably be constructed with AG MP-50 in place of MSC-1 and with AG MP-1 in place of A-162.

Eluants For Ion Exchange Resins

Any formulation of the parfait method must solve the problem of recovering the organic solutes exchanged onto the ion exchange beds and separating them from the ions in the eluant. Since high-ionic-strength eluants are typically used to recover exchanged ions, the problem of separating traces of organic solutes from the eluting ions can be a difficult one. In the original parfait method, the separation was accomplished by using a volatile buffer, 2 M triethylammonium

TABLE 15. POLYSTYRENE ION EXCHANGE RESINS CONSIDERED FOR USE IN THE PARFAIT SYSTEM

Resin	Supplier	Type[a]	Manufacturer's stated capacity (meq/ml) wet
AG MP-1	BioRad	S.B., MA	-
AG MP-50	BioRad	S.A., MA	1.7
IR 120	Mallinckrodt	S.A., MI	1.9
IR 252	Mallinckrodt	S.A., MA	-
IRA 400	Mallinckrodt	S.B., MI	1.4
IRA 900	Mallinckrodt	S.B., MA	1.0
Duolite A-162	Diamond Shamrock	S.B., MA	1.0
Dowex MSC-1	Sigma	S.A., MA	2.0

[a] S.A. = strong acid; S.B. = strong base; MA = macroporous; MI = microporous.

bicarbonate pH 7.5, to elute the bed. When such an eluate was lyophilized, the eluting ions equilibrated with their neutral forms, which volatilized as triethylamine and CO_2, leaving any poorly volatile solutes from the sample in the residue.

Volatile buffers were reconsidered in the current study. Triethylamine was ruled out primarily because it could not be obtained in a highly purified form and because the secondary and primary amines contaminating it could potentially react with solutes present in the water sample. Preliminary evidence of reaction between ethidium bromide and triethylammonium bicarbonate was obtained, but the reaction product was not characterized. The components of volatile buffers that appeared acceptable on the basis of chemical purity were ammonia, acetic acid, and formic acid. A few exploratory experiments were conducted involving the elution by ammonium formate and ammonium acetate of EB or quinaldic acid exchanged onto AG MP-50 or IRA 900. These experiments showed that 1 M ammonium formate in water was a very poor eluant, but that EB could be eluted from AG MP-50 with 1 M ammonium formate in methanol. Elution was essentially complete with six bed volumes of the methanolic eluant, whereas neither methanol alone nor aqueous 1 M ammonium formate was able to elute this solute. This again pointed out the necessity for a counterion to displace exchanged solutes and, additionally, indicated the importance that the displaced solute be highly soluble in the solvent carrying the eluting counterions.

UV-absorbing material, considered to be quinaldic acid, was readily eluted by 1 M ammonium acetate in methanol. However, controls for this experiment showed that a considerable amount of UV-absorbing material could be eluted from columns that had not been loaded with quinaldate; therefore, firm conclusions about the suitability of ammonium acetate could not be made. Glacial acetic acid was also briefly considered in this series of experiments, with results similar to those with ammonium acetate.

Only later in the study, after MSC-1 and A-162 were chosen as the ion exchange resins, was the choice of an eluting solvent returned to. By this time, the liberation of contaminating materials from the resin by the eluting solvent was recognized as the most serious question to be addressed, and porous Teflon had been chosen as the first bed in the parfait column. Thus we expected that all poorly water-soluble solutes would adsorb to Teflon and that none of them would reach the polystyrene-divinyl benzene matrix of the ion exchange resins. Therefore, the elution of the ion exchange resins could focus exclusively on the exchanged ionic solutes.

The approach tested was to neutralize the H^+ or OH^- counterions on the bed, thereby converting weak organic acids or bases to their neutral form, and to elute with organic solvents, in which such neutral organics should be readily soluble. We expected this approach to have the advantages of exposing the resins to lower-ionic-strength eluants, which might reduce the liberation of contaminants from the resin support matrix, the advantage of selectively recovering the organic solutes in a neutral, organic-soluble form, separate from inorganic ions, and the advantage of recovering the eluted solutes in a medium easily concentrated in a Kuderna-Danish apparatus, in anticipation of analysis by gas chromotography. Full advantage depended upon the exclusion of water from the eluting solvent.

Experiments began using 12% methanol in methylene chloride (solvent 1), a solvent that readily dissolves molar concentrations of anhydrous HCl or NH_3 gas. The apparatus used to introduce these gases into solvent 1 to prepare the eluants was shown in Figure 1 (Section 4, above). In general, a concentrated stock of HCl or NH_3 in solvent 1 was prepared and its concentration determined by titration. The stock was diluted with fresh solvent 1 to obtain the desired acid or ammonia concentrations. All tubing used in this apparatus must be Teflon, or phthalate esters and other contaminants will occur in the eluates, and the solvents must be protected from moisture by admitting air only through drying tubes containing $CaSO_4$.

Development of the Protocol for Eluting the Ion Exchange Resins

In the first series of experiments to develop an elution protocol, beds of resins were tested and the eluates concentrated to 1 ml in a Kuderna-Danish apparatus under a 3-ball Snyder column. Unless otherwise indicated, these concentrates were methylated with diazomethane, and the

methylated solutes analyzed by gas chromatography. The first experiments focused on A-162. Although this resin apparently contributed fewer contaminants to organic solvent eluates than did the other macroreticular strong anion exchange resins, it was noticeably worse than comparable MSC-1 or AG MP-50 eluates. Thus, it was a suitable test system for development of the protocol. (All of the resins tested in the following experiments were cleaned according to the protocol described by Junk et al. (1974) unless otherwise indicated. They were stored in methanol and charged with OH^- [A-162] or H^+ [MSC-1] just before use.)

In the first experiment, 200 ml of A-162 was placed in an addition funnel and eluted with one bed volume of 1.0 N HCl in solvent 1, collected in 50-ml increments; followed by four bed volumes of 0.10 N HCl in solvent 1, collected in 200-ml increments; and four bed volumes of methanol, collected in 200-ml increments. The Kuderna-Danish concentrates of the first fractions only reduced to about 2 ml and wouldn't reduce further, apparently because of residual water. The first fraction was as strongly colored as weak tea, and the second and subsequent fractions were progressively more strongly colored. The concentrates from the 0.10 N eluate reversed this trend, becoming essentially colorless by the fourth bed volume. The first methanol eluate was colored but the remaining three were colorless. The contamination of the concentrated eluates was so evident visually that it was not verified instrumentally. This experiment showed that it was the shock of the eluant that released the contaminants, because concentrates of water passed through comparable beds of the cleaned resin were colorless.

In the next experiment, 75 ml of A-162 was exposed to 8 l of unspiked synthetic hard water to simulate a typical parfait experiment. The resin was allowed to run dry by gravity, water was expelled by a gentle flow of N_2, and the resin was then dried with four bed volumes of absolute methanol, two of absolute ethanol, and one more of methanol. The last bed volume of methanol was collected and concentrated, yielding about 2 ml of a solution boiling at 99°C (i.e., probably a water-alcohol mixture). An additional bed volume of methanol yielded only about 0.1 ml of the mixture. After one more bed volume of methanol, the resin was considered to be dry. The resin was then eluted with one bed volume of 1.0 N HCl in solvent 1, four bed volumes of 0.10 N HCl in solvent 1, and four bed volumes of methanol. The first solvent produced a colored eluate. Eluates were essentially colorless by the end of the second solvent. Concentrates of the methanol eluate were colorless and free of peaks detectable by GC, except for a single broad, late peak. The inability of the dehydrating alcohols to prevent contamination of the initial acid-solvent 1 eluate again suggested that the contaminants were not simply adsorbed onto the resin surface, but released by the shock associated with exposure to the eluant. Also, the hydration-dehydration cycle did not, by itself, seem responsible for the contamination.

The next experiment attempted to define the component of the eluant responsible for the release of contaminants. A 50-ml bed of A-162 was eluted with one bed volume of solvent 1, followed by three successive bed volumes of 0.33 N HCl in solvent 1, then two bed volumes of 0.50 N HCl in solvent 1, then two successive bed volumes of 1.0 N HCl in solvent 1. This was followed by 100 ml methanol, 100 ml H_2O, 100 ml methanol, and 100 ml of 0.50 N HCl in water. The first eluant (solvent 1) produced a colorless concentrate; the next three fractions of 0.33 N HCl in solvent 1 were yellowish, but the following two eluants of successively higher acid concentration in solvent 1 were all colorless. No color was elicited by the elutions with alternating methanol-water or methanol-acid. The first conclusion from this experiment is that the acid in solvent 1 was responsible for the shock that produced the contamination. The failure of increasing acid concentrations in solvent 1, or aqueous acid, to release more contamination suggested that the acid shock was only effective when the acid occurred in organic solvents, although the results could also be explained if the first shock had removed all available contaminants, so that no more release was possible in the subsequent steps.

The significance of the acid concentration was explored by exposing three separate 50-ml beds of A-162 to different eluants. The first bed was exposed to three bed volumes of solvent 1, followed by three bed volumes of 0.33 N HCl in solvent 1 and then one bed volume of 0.10 N HCl in solvent 1. The second bed was directly eluted with three bed volumes of 0.33 N HCl. The third was eluted with two bed volumes of 0.50 N HCl in solvent 1. The color produced in concentrates of the acidic eluates increased from the first to second to third bed tested; that is, decreasing acid concentration reduced contamination, and acclimating the resin to solvent 1 also reduced contamination. The concentrate of the 0.10 N HCl eluate of the first bed was colorless.

A final exploratory experiment employed 75 ml of A-162 exposed to 8 l of synthetic hard water. This resin was first dried with four bed volumes of methanol, followed by two bed volumes of absolute ethanol and then four more bed volumes of methanol. These eluates were discarded. Elution began with two bed volumes of solvent 1, followed by two bed volumes of 0.33 N HCl in solvent 1 and then four bed volumes of 0.10 N HCl in solvent 1. Only the concentrate of the 0.33 N HCl eluate showed color; it was pale yellow. Based on these results the cleanup of the resin was modified to that described in Section 4 (Materials and Methods).

The experience with A-162 led to a similar cleanup and elution sequence with MSC-1, except that anhydrous NH_3 was substituted for HCl in solvent 1. Unlike the A-162 eluates, the MSC-1 eluates did not visibly differ in contamination. Therefore, these were combined and concentrated as one solution. A-162 eluates were kept as separate fractions, individually concentrated and analyzed, both because they differ in their levels of background contamination (the 0.33 N HCl-solvent 1 eluate being the most contaminated) and because solutes

tended to distribute themselves among the eluates, as described in subsequent sections.

Elution of Solutes from A-162

During development of the elution procedures, parallel attempts were made to assess the recovery of organic acids adsorbed to A-162. Usually, these experiments were thwarted by the contaminants that appeared in the GC profiles and interfered with the quantitation of the test solutes. With the development of the protocol just described, elution of model solutes was again assessed. A pair of 75-ml beds of A-162 charged with NaOH were exposed to 8 l of synthetic hard water either spiked with 400 g each of benzoic acid, o-phthalic acid, quinaldic acid, and trimesic acid (column 94) or unspiked (column 93). After being dryed with alcohols as described, each column was eluted with two bed volumes of solvent 1, two bed volumes of 0.33 N HCl in solvent 1 (fraction A), four bed volumes of 0.10 N HCl in solvent 1 (fraction B), and four bed volumes of methanol (fraction C). Table 16 shows the recoveries of these acids.

Recovery of the solutes tested above was complicated by the tendency of A-162 to float in solvent 1. Thus, it was possible that not all of the A-162 surface was consistently bathed in solvent during elution. To remedy this problem, we substituted a 60-ml addition funnel for the 150-ml funnel previously used and packed it with 65 ml of A-162, compressing the resin gently at the top. In this manner, the resin was held in place during elution, guaranteeing uniform wetting of the resin's surface with solvent. Again 400 µg each of the four organic acids in 8 l of synthetic hard water was applied to one bed (column 96), and unspiked hard water to a control bed (column 95). After the column was dried with alcohols and conditioned with solvent 1, fractions A (150 ml 0.33 N HCl in solvent 1), B (four bed volumes of 0.10 N HCl in solvent 1), C (four bed volumes of methanol), and D (four bed volumes of 0.10 N HCl in methanol) were collected, concentrated, methylated, and analyzed. Table 17 shows the recoveries. The containment of the resin produced much better recoveries of phthalic and trimesic acid. In contrast, quinaldic acid was not found. Quantitation of benzoic acid was not possible because of the coelution of a major impurity.

The relatively great recovery of trimesic and phthalic acids in fraction D suggested either that these acids were much more soluble in methanol than in solvent 1 or that fractions A and B provided insufficient acid to neutralize the ion exchange resin and thereby liberate the acids into the solvent. The manufacturer's value for the exchange capacity of A-162 was 1 meq/ml, suggesting that 65 meq was needed to neutralize the resin. Fraction A provided only 50 meq and fraction B an additional 26 meq, values consistent with the first hypothesis. Direct measurement of the capacity of A-162 gave a value of 0.86 meq/ml, indicating that only 56 meq would be needed to neutralize the bed.

TABLE 16. PERCENT RECOVERY OF ORGANIC ACIDS FROM A-162 (COLUMNS 93 AND 94)[a]

	Benzoic acid	o-Phthalic acid	Quinaldic acid	Trimesic acid
Fraction A	35.1	NF	NF	NF
B	TR	10.3	16.4	16.7
C	58.9	5.7	NF	6.7
Total	94.0	16.0	16.4	23.4

[a] NF = not found; TR = trace.

TABLE 17. PERCENT RECOVERY OF ORGANIC ACIDS FROM A-162 (COLUMNS 95 AND 96)[a]

	o-Phthalic acid	Quinaldic acid	Trimesic acid
Fraction A	NF	NF	16.9
B	NF	NF	7.8
C	NF	NF	12.5
D	48.4	NF	35.3
Total	48.4	0	72.5

[a] NF = not found.

To settle the question, 65 ml of A-162 was packed and exposed as before to spiked and unspiked water (columns 97 and 98). After the column was dried and acclimated to solvent 1, elution yielded fraction A (180 ml 0.33 N HCl in solvent 1), B (four bed volumes of 0.10 N HCl in solvent 1), C (four bed volumes of methanol), and D (four bed volumes of 0.10 N HCl in methanol). Phthalic acid was not recovered in fraction A; 26.4% was detected in B, 18.0% in C, and none in D, for a total of 44.4% recovered. None of the other acids were quantifiable, because of co-eluting impurities. From the results with phthalate, it appears that the previous columns were incompletely neutralized.

To confirm this interpretation, columns 99 and 100 (65 ml bed volumes each) were set up, exposed to quinaldic, phthalic, and trimesic acids, and dried and conditioned as before. Each was then eluted with 195 ml 0.33 N HCl in solvent 1 (A), three successive four-bed-volume aliquots of 0.10 N HCl in solvent 1 (B, C, D), and three successive four-bed-volume aliquots of methanol (E, F, G). Table 18 shows the recoveries obtained.

Several conclusions can be drawn from these experiments. Clearly, the acidic methanol is important for the elution of compounds like trimesic acid. Also, the recovery of quinaldic acid is erratic. This is discussed in a later section.

TABLE 18. RECOVERY OF ORGANIC ACIDS FROM A-162 (COLUMNS 99 AND 100)[a]

% Recovered	o-Phthalic acid	Quinaldic acid	Trimesic acid
Fraction A	14.5	NF	4.8
B	13.8	NF	1.4
C	8.6	NF	2.0
D	8.7	NF	2.2
E	24.0	NF	23.8
F	2.3	1.5	5.1
G	NF	NF	4.0
Total	71.9	1.5	43.3

[a] NF = not found.

The results further indicate that the elution method developed here is a compromise, balancing the contamination of the eluate, especially fraction A, against an incomplete recovery of solutes like trimesic acid, which tend to be released from the resin with difficulty. However near-quantitative recoveries of simpler acids, like benzoic and phthalic acids can apparently be expected from this protocol. The protocol has the advantage of selectively recovering weak acids separately from strong anions, and it avoids the use of high concentrations of strong eluting ions.

The elution scheme developed here might not be suited to all end uses of the recovered compounds. Because the solutes are exposed to methanol in the presence of anhydrous HCl, methyl esters may form. For eventual analysis by GC or GC/MS, this poses no problem, because these concentrates are methylated prior to analysis. However, if bioassay is the intended end use, the extent of methylation should be considered and alternative elutions, perhaps utilizing volatile buffers, might be developed.

The elution protocol developed here was responsive to several constraints, particularly the need for an eluate clean enough for quantitative analysis of the model solutes. It grew from a preconceived strategy, namely to condition the exchange resin for elution with its solutes still attached, by denying the solutes a mobile counterion, and then selectively titrating and eluting the weak acids (or bases) into organic solvents. In the end, the potential for elution of the ion exchange beds with pure volatile buffers was not fully explored, and this approach may yet offer some useful and acceptable means of recovering organic solutes from ion exchange beds.

Methylation of Trimesic Acid and 5-Chlorouracil

Variations in the efficiency of methylation of the organic acids used to develop the elution protocol for A-162 could account for the variable recoveries seen. If, for instance, methylation of the standard solutions were markedly more efficient than methylation of the experimental concentrates, then spuriously low recoveries would be calculated.

To test the influence of the solvents in which methylation took place, two sets of standard solutions were made of the four acids tested above, one in methanol and one in solvent 1. Aliquots of the standard in solvent 1 were diluted into three 100-ml volumes of 0.33 N HCl in solvent 1, concentrated by the usual protocol, methylated, and adjusted to 1.0 ml before GC analysis. A similar series was carried out by diluting the methanol standard into 10-ml volumes of methanol. The curves of peak area for each acid versus the amount of standard originally diluted into solvent matched when superimposed. Thus, solvent had no influence on the efficiency of the methylation.

This experiment revealed that quinaldic acid was poorly methylated relative to the carboxylic acids. It is well known that diazomethane methylation does not work equally well with all solutes (Keith, 1977). We decided to explore some alternative methods to make volatile derivatives of the organic acids and bases that are difficult to quantify by GC.

One approach examined was the formation of methyl esters by the pyrolytic decomposition of the tetramethylammonium salts of the solute during injection into a gas chromatograph (Robb and Westbrook, 1963; MacGee, 1966). This approach was tested with both trimesic acid and 5-chlorouracil, using injector port temperatures in the range of 225°-300°C. At all temperatures tested, trimesic acid gave multiple peaks in the chromatogram, constituting no improvement over the diazomethane method. The 5-chlorouracil salt gave one principal peak at an injector port temperature of 240°C. However, when the concentration of the injected material was reduced to the standard concentration of 400 µg/ml, no detectable peaks were observed, suggesting either that the methylation reaction or the detector sensitivity was inadequate at this concentration.

One other attempt was made to prepare a gas-chromatographable derivative of 5-chlorouracil. Silylation by bis(trimethylsilyl) acetamide was attempted, based on the protocol of Gehrke et al. (1967). Again, no peak corresponding to a 5-chlorouracil derivative was detected when 400 µg/ml standard was used.

Interferences Associated with Eluates

Several sources of interferences and errors were possible in the preceding work and the recoveries reported in the next section. For instance, interfering contaminants appeared in most of the eluates. Consequently, blank (unspiked) columns were routinely run in parallel with each spiked sample, and only peaks that could be unequivocally ascribed to the spiked solute were quantified. The following attempts were made to identify the source of the contaminants.

In addition to the experiments described in a previous section, a study was made of the eluting solvent itself. In one such experiment, 400 ml of methylene chloride (A), 150 ml of 0.33 N NH_3 in solvent 1 plus 120 ml of 0.10 N NH_3 in solvent 1 plus 120 ml of methanol (B), 150 ml of 0.33 N HCl in solvent 1 (C), solution B plus 13.8 mg of bis(2-ethylhexylphthalate) (D), and 260 ml of 0.10 N HCl in methanol (E) were concentrated to 1 ml in a Kuderna-Danish apparatus and under N_2, according to the usual protocol for column eluates. Gas chromatography of these concentrates showed A to be essentially uncontaminated; B, D, and E were moderately contaminated and strongly resembled the eluates from the A-162 blank columns used in the preceding section, and C was extremely contaminated. When rechromatographed on a freshly packed column, only C showed a cleaner profile.

This experiment established that the eluting solvent itself was a source of contaminants, and it suggested that concentrates of the acidic eluates could in addition elute materials from the injection port or the early section of the gas chromatography column packing. Decomposition of phthalate esters during concentration did not appear to be a problem.

The major contaminants in the eluates of blank column 115 were identified by GC/MS. The MSC-1 fraction (F2) and the first, acidic fraction from the A-162 resin (F3) were analyzed in a Hewlett-Packard Model 5985 GC/MS, using a temperature program of 100°C for 5 min, then a linear temperature increase of 4°C/min to 280°C. Compounds were identified by peak matching with reference to an EPA library of mass spectra and the 8-peak index.

The MSC-1 eluate contained eight major contaminants. Methylhexadecanoate, methylbutylphthalate, methylstearate, methyl-4-methyldodecanoate, dioctyl phthalate (two peaks), and an alkyl benzene were identified. The eighth peak (the fourth to elute) corresponded to the spectrum of an isomer of geranyl citronellal [$(H_3C)_2C = CHCH_2CH_2C(CH_3) = CHCH_2CH_2C(CH_3) = CHCH_2CH_2C(CH_3)CH_2CHO$]. The A-162 eluate produced only one major peak, which was confirmed by analysis of a standard as bis(2-ethylhexyl)phthalate.

These two experiments pointed to plasticizers as the contaminants in the eluting solvents. Although plastic tubing had been avoided in all of the parfait column operations, we then realized that a short section of tygon tubing was in fact connecting the anhydrous NH_3 and HCl bottles to the solvent in the eluting solvent generator (Figure 1). When this was replaced with Teflon tubing, the profile from F2 was improved, although it was still not without contaminants. Figures 10, 11, and 12 show the GC profiles of various eluates of blank parfait columns, solute standards, and solutes recovered from a spiked column. These figures show the results typical of the best chromatograms obtained during the study. Among them, the least acceptable profiles were obtained from F3, the concentrate of the most acidic methanol-methylene chloride solvent, the one that induces the greatest shock to the A-162 support matrix. The only precaution that was found to reduce contamination of the GC profile of this eluate was to use a freshly packed Carbowax phase for chromatography support.

Finally, concentrates of parfait columns were routinely assayed within 48 h of preparation. Evidence for slow evaporation of F1 was obtained, even when Supelco Teflon-lined septum vials were used. Individual concentrates were rechromatographed at various times, and it appeared that profiles could not be reliably reproduced after several days, even when the concentrates were stored at -20°C. Certain compounds, particularly BHT and furfural, were found to change their profiles in a matter of days, probably due to decomposition.

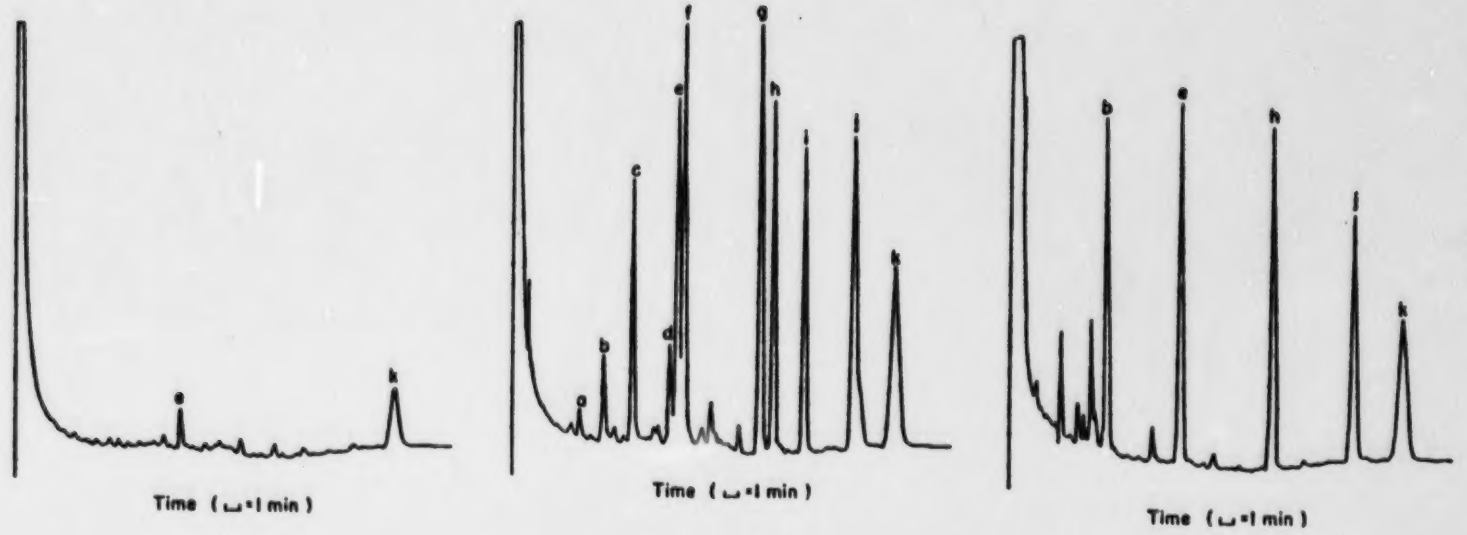

Figure 10. Gas chromatograms of parfait fraction F1 from an unspiked control column (left panel), column 120 (middle panel), and one of the standards used to calibrate the chromatogram (right panel). Designated solutes are a = furfural, b = isophorone, c = 1-chlorododecane, d = quinoline, e = BHT, f = biphenyl, g = 2,4-dichlorobiphenyl, h = stearic acid, i = 2,2',5,5'-tetrachlorobiphenyl, j = anthraquinone, and k = bis(2-ethylhexyl)phthalate.

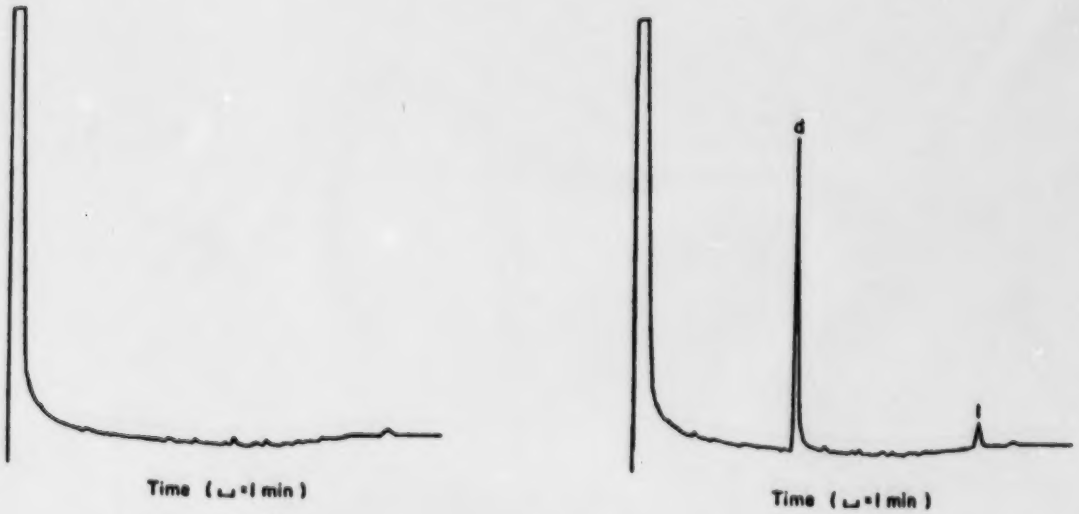

Figure 11. Gas chromatograms of parfait fraction F2 from an unspiked control column (left panel) and column 120 (right panel). Designated compounds are d = quinoline and 1 = caffeine.

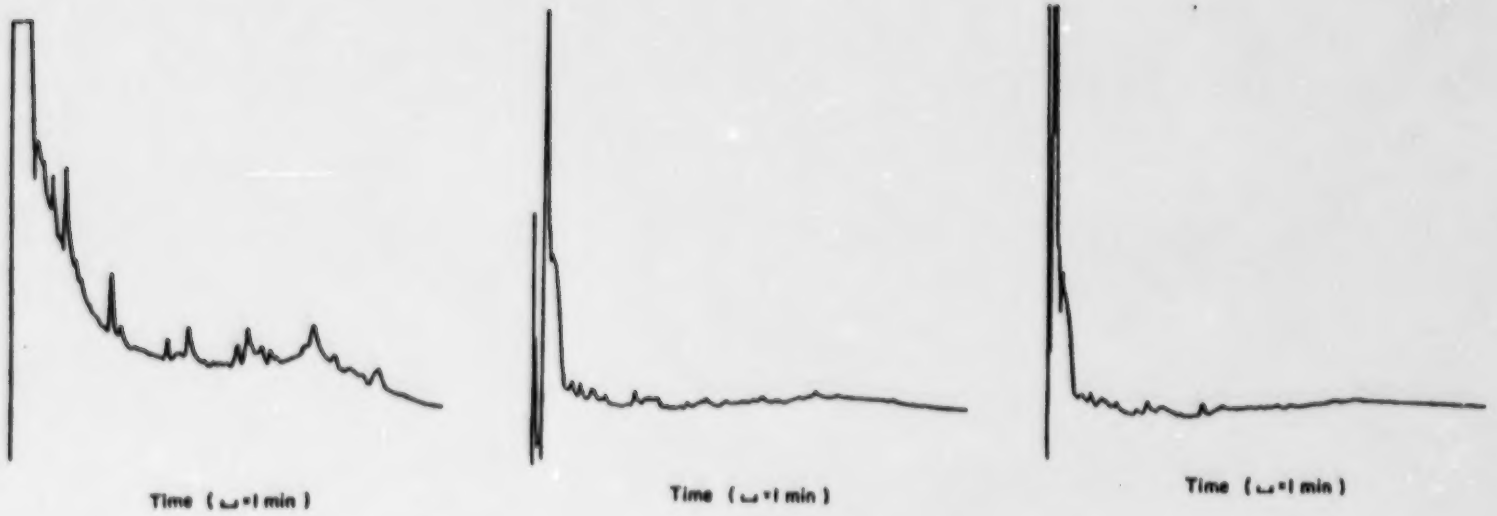

Figure 12. Gas chromatograms of parfait column fractions F3 (left), F4 (middle), and F5 (right) from an unspiked control column.

HUMIC ACID

A solution of 16.0 mg/ml Fluka humic acid was prepared by adding 1 M NaOH dropwise to a water slurry of the solid in a volumetric flask until the acid was dissolved. Dilutions of this concentrate were used to make 2.0 mg/l solutions of the humate in synthetic hard water.

The pH of the humic acid solutions behaved as expected for polymers containing weakly acidic groups. The pH of the 16-mg/ml stock, as detected by a combination glass-reference electrode, changed slowly and did not stabilize. A typical value for the pH after 30 min of stirring was 4.5. When the humic solution was supplemented with 0.5 M KCl to aid the equilibration of protons with titratable groups, the pH came to a stable value after a few minutes. A pH of 3.9 was then recorded. As the humic acid solution was progressively diluted to 20 µg/ml in 0.5 M KCl, the equilibrium pH rose to 6.4. The pH of the humic acid diluted to 2.0 µg/ml in the synthetic hard water was stable and did not differ detectably from the pH of the water before addition of the humate. This was undoubtedly due to the buffer capacity and availability of ions in this water.

Humic acid did not contribute detectable impurities to the standard eluates of blank parfait columns. When humic acid was applied to a parfait column having beds of 50 ml PTFE, 30 ml MSC-1, and 65 ml A-162, the PTFE bed became strongly colored by the humate, the A-162 showed some coloration, and the column effluent also contained humic acid. Extraction and analysis of the two ion exchange beds by the standard protocol did not reveal any detectable impurities, apparently because the humic acid was insoluble in the solvents used, and any humic acid that may have been extracted could not volatilize in the gas chromatograph. When the PTFE fraction was extracted with methylene chloride, the residual aqueous solution that separated from the eluate was strongly colored, but no color was apparent in the methylene chloride phase, nor were discernable contaminants contributed to the GC pattern of this concentrate.

The experiment above suggested that porous PTFE had a high capacity for humic acid, but that the amount of humic acid applied (16 mg total) had saturated the bed. In a subsequent experiment, 8-l of 2.0 ppm humic acid was again applied to a parfait column, but this time three sequential 50-ml beds of Chromosorb T preceded the cation and anion exchange beds. In this column, the amount of humate recovered in the aqueous residue from methylene chloride elution of each Teflon bed and the humate in the column effluent were estimated from the absorbance at 200 nm and volume of each of these subfractions. Of the total humate applied to the column, 8.9, 5.0, 2.9, and 5.1% were recovered in the aqueous residue of the three Teflon beds and the column effluent, respectively. No attempt was made to deliberately recover the humate from the Teflon or the ion exchange beds, but it was clear from the color remaining on the Teflon that a great deal of material remained uneluted. Based on the results of this experiment, a 150-ml bed of

Chromosorb T was used in all parfait columns from no. 110 onward. This amount of Teflon is sufficient to adsorb between 90 and 95% of the 16 mg of Fluka humate applied.

RECOVERY OF TEST SOLUTES

The most remarkable finding regarding humic acid was its failure to adversely affect the recovery of the test solutes. The results in Tables 19 and 20 show no significant interference in the recovery of the test solutes, except perhaps for caffeine and 2,4-dichlorophenol. In the cases of 2,2′,5,5′-tetrachlorobiphenyl, bis(2-ethylhexyl)phthalate, 1-chlorododecane, biphenyl, and anthraquinone, the columns containing humic acid averaged a higher recovery than those lacking it. However, this was probably due to the use of a 150-ml bed of Teflon in the latter columns rather than to the presence of the humic acid. The tetrachlorobiphenyl, chlorododecane, and biphenyl were also tested on a pair of columns with a single 50-ml bed of Teflon and lacking (column 108) or containing (column 109) humic acid. A high recovery was seen in this pair of columns, but not as high as was seen in the columns using the 150-ml Teflon bed and containing humate (columns 112-120).

A similar comparison can be made with caffeine on columns 108 and 109, where recovery decreased about 20% in the presence of the humate. In this case, however, caffeine was not recovered from the Teflon fraction of columns 117, 118, and 120, which used the larger bed of Teflon. It is possible that the larger amount of humic acid collected on the 150-ml Teflon bed influenced the partition of caffeine between the water and methylene chloride phases in the eluate. It was previously noted that substituted xanthines like caffeine showed an anomalously high adsorption to Teflon, relative to their water solubility, possibly because their water solubility was enhanced by the formation of oligomers in solution (Guttman and Higuchi, 1957; Cesaro et al., 1980). The results in Table 20 could be rationalized if humic acid significantly enhanced the solubility of caffeine in the aqueous phase created by elution of Teflon. Aromatic compounds including phthalate esters and DDT have been found to associate with dissolved humic acids (Carter and Suffet, 1982; Matsuda and Schnitzer, 1971), although it is not known if caffeine behaves in this way. Simple comparisons of the partition of caffeine between methylene chloride and water or methylene chloride and humate solutions would test this possiblity. It is difficult to suggest where the caffeine went without postulating such an effect.

The only other effect possibly attributable solely to humic acid involved 2,4-dichlorophenol, the most broadly distributed compound tested in this study. In columns 108 and 109, the humic acid apparently decreased the ease of elution of the chlorophenols, since lower overall recoveries were obtained from column 109, which included the humate, than in 108, which was humate-free. Also, recovery was detected in F6 of column 109 but not of column 108. This also suggests that humate

TABLE 19. RECOVERY OF COMPOUNDS FOUND EXCLUSIVELY ON TEFLON

Compound[a]	% recovery on column number[b]								
	106	108	109	112	114	117	118	120	Mean[c] ± S.D.
Stearic acid	105.					106.	93.6	95.6	98.4 ± 6.6
2,4'-Dichlorobiphenyl	73.8			72.3		81.5	75.1	80.6	79.1 ± 3.5
2,2',5,5'-Tetrachlorobiphenyl		69.0	77.0			85.1	80.0	89.1	84.7 ± 4.6
Bis(2-ethylhexyl)phthalate	61.6					104.	100.	78.8	94.3 ± 13.5
1-Chlorododecane		29.7	37.6			70.6	62.4	78.3	70.4 ± 8.0
Biphenyl		49.0	53.5			90.1	80.7	96.8	89.2 ± 8.1
Anthraquinone	65.8					107.	102.	119.	109. ± 9.
2,6-Di-tert-butyl-4-methylphenol (BHT)	129.					103.	89.1	82.8	91.6 ± 10.3
Methyl isobutyl ketone					47.8				

[a] All compounds were exposed to the parfait column in 50 ppb concentration in synthetic hard water.

[b] Columns 109-120 were also spiked with 2.0 mg/l humic acid. Columns 106-109 had a 50-ml bed of Chromosorb T; columns 112-120 had a 150-ml bed of Chromosorb T.

[c] Means only include data from columns 117, 118, and 120.

TABLE 20. PERCENT RECOVERIES OF TEST SOLUTES BY FRACTION [a]

Trimesic Acid					
Column no.	F3	F4	F5	F6	Total
102	7.6	6.8	41. [b]	15.2	70.6
104	4.8	4.3	19.2	6.9	35.2
106	7.1	7.1	19.0	6.9	35.2
112	4.9	6.1	40.2	24.4	75.6
117	NF	1.2	14.9	9.3	25.4
118	3.1	2.5	20.5	9.3	35.4
120	TR	1.5	6.4	NF	7.9
Mean ± S.D. [b]	1.0 ± 1.8	1.7 ± 0.7	13.9 ± 7.1	6.2 ± 5.4	22.9 ± 13.9

Isophorone					
Column no.	F1	F2	F3	F7	Total
114	35.8	1.5	0.6	5.0	42.9
117	20.3	TR	TR	TR	20.3
118	19.8	TR	TR	TR	19.8
120	35.7	NF	NF	NF	35.7
Mean ± S.D.	25.3 ± 9.0				25.3 ± 9.0

Caffeine				
Column no.	F1	F2	F7	Total
108	15.9	33.4	51.1	100.4
109	15.3	32.5	35.0	82.8
117	NF	7.4	26.4	33.8
118	NF	9.8	33.0	42.8
120	NF	8.1	NF	8.1
Mean ± S.D.	NF	8.4 ± 1.2	19.8 ± 17.5	28.2 ± 18.0

(continued)

TABLE 20, continued.

2,4-Dichlorophenol

Column no.	F1	F2	F3	F4	F5	F6	Total
108	2.3	12.9	31.6	34.5	4.6	TR	85.9
109	1.7	8.9	7.0	5.8	5.8	4.7	33.2
117	3.9	NF	28.1	18.8	10.9	14.1	75.8
118	5.5	NF	10.9	18.0	7.8	11.7	53.9
120	NF	NF	19.8	4.0	1.0	NF	24.8
Mean ± S.D.	3.1 ± 2.8	NF	19.6 ± 8.6	13.6 ± 8.3	6.6 ± 5.1	8.6 ± 7.5	51.5 ± 25.6

Furfural

Column no.	F1	F3	F4	F5	F6	Total
114	NF	11.7	26.3	NF	NF	38.0
117	29.5	1.0	NF	NF	NF	30.5
118	6.1	16.3	NF	NF	3.7	26.1
120	22.8	10.6	9.9	7.0	TR	50.3
Mean ± S.D.	19.5 ± 12.0	9.3 ± 7.7	3.3 ± 5.7	2.3 ± 4.0	1.2 ± 2.1	35.6 ± 12.9

Quinoline

Column no.	F2
MSC-1 #2	57.
104	80.8
112	46.6
117	46.3
118	51.3
120	111.
Mean ± S.D.	69.4 ± 35.8

(continued)

TABLE 20, continued.

Glucose Column no.	F7
106	56.4
107	119.
110	97.5
117	89.4
118	67.8
120	105.
Mean ± S.D.	87.4 ± 18.7

[a] TR = trace; NF = not found.

[b] All means include columns 117, 118, and 120 only.

enhanced binding of the phenol to the column. The reproducibility of 2,4-dichlorophenol recovery among the various parfait fractions was poor, as is illustrated by the results from replicate columns 117, 118, and 120. Because of this variability, the differences in its recovery in columns 108 and 109 may reflect the reproducibility of the method rather than the influence of the humate.

Glycine and Quinaldic Acid

Two solutes were not accounted for satisfactorily in this study, quinaldic acid and glycine. Although more experiments were performed involving these two solutes than any others, most of these data cannot be interpreted unequivocally. Analytical problems, impurities eluting from the ion exchange resins, and unrecognized sources of loss account for this situation.

Several specific errors were discovered in these experiments. In early work, quinaldic acid was quantified by its optical absorbance at 248 nm. Impurities leaching from the ion exchange resins contributed variable and unquantified background absorbance, a situation that wasn't recognized until experiments were performed in which apparent recoveries of 1500% of the applied quinaldate were obtained. In retrospect, the amount of UV-absorbing impurity appearing in these eluates depended upon the resin, the resin cleanup protocol, and the eluant. As described in the previous section, organic solvents containing acids or bases induced the greatest levels of contamination.

When quinaldic acid was quantified by gas chromatography in later experiments, the apparent recoveries were always quite low. A low yield of chromatographable product during methylation and interference with the chromatography by water probably accounted for this result. As mentioned in the previous section, when a standard solution containing equal weights of benzoic, trimesic, phthalic, and quinaldic acids was methylated, the peak areas were much smaller for quinaldic acid than for the other acids. In one experiment, for instance, the methylated product from an original 400 ng of benzoic acid gave a peak area of 1.04×10^6 units, 400 ng of trimesic acid gave 1.29×10^6 units, but 400 ng of quinaldic acid in the same solution only gave 1.01×10^5 units.

These peak areas were sufficient to quantify quinaldic acid when Carbowax columns were used, but residual water present in the extracts of the ion exchange resins apparently interfered when the polydimethylsiloxane stationary phase was used. Silicone phases were susceptible to hydration, which caused decreased resolution and baseline drift. The change to a polyethylene glycol stationary phase eliminated the water interferences, and good linear calibration curves for methyl quinaldate were then obtained. It was at about this time that the standard elution protocols were adopted and the recovery studies described in the next section were performed.

It was a surprise when these recovery studies detected only traces of quinaldic acid (columns 112 and 122). The traces occurred in fractions F5 and F6, the methanolic eluates of the anion exchange resin. Over 95% of the quinaldic acid was not found.

Biodegradation was rejected as an explanation for the loss of the quinaldic acid. First it was recalled that eluates of parfait beds consisted of anhydrous neutral acidic or basic organic solvents, all inimical to microbial life. Second, it was noted that no other solutes, except glycine, were lost from the parfait system in substantial amounts. This included readily metabolized solutes such as stearic acid, caffeine, and glucose. In columns 112 and 122 substantial recoveries of the solutes accompanying quinaldic were obtained. In the preliminary experiments reported in Tables 16, 17, and 18, the aromatic acids accompanying quinaldate also were recovered in substantial amount, but the quinaldate was not. Biodegradation was most likely in the F7, because this fraction did not contact bacteriostatic agents unlike the fractions obtained from the parfait column beds. However, glucose, the most readily biodegraded solute tested in these experiments, was recovered virtually quantitatively from this fraction. To explain the loss of quinaldate through biodegradation, it would be necessary to postulate the selective biodegradation of this compound in the presence of equally available and susceptible materials, often under physical conditions inhibitory to microbial activity. This seemed highly unlikely.

It is possible that zwitterions could be lost from the parfait method if they were soluble in the alcohols used to dehydrate the ion exchange beds. Usually compounds can be eluted from a bed only if they are provided with a mobile counterion or if they can be converted to a neutral species by titration with acid or base. Zwitterions, however, can be associated with an ion exchange bed without actually exchanging for the bed's original mobile counterion. For example, if quinaldic acid enters a cation exchange bed in the H^+ form, the low pH prevalent there may both neutralize quinaldic's carboxylic acid function and protonate its aromatic nitrogen, creating a cation associated with the ion exchange bed's stationary anion, but not actually exchanged for the original proton counterion. When the bed is neutralized by ammonia, the protonated quinaldinium ion will be converted to anionic quinaldate and it will elute if it is soluble in the mobile phase. But if the bed is first washed with an ion-free solvent such as ethyl alcohol, the quinaldinium can, in principle, dissociate into neutral quinaldic acid and a proton, and the neutral acid may be eluted in the alcohol. Quinaldic acid is soluble in alcohol (Merck Index, 1975). In the lab, several milligrams of quinaldic acid dissolved rapidly in 1 ml of absolute methanol. Thus, it is at least possible that this series of events accounts for the virtually quantitative disappearance of quinaldic acid from the parfait system. This interpretation is consistent with the data in Tables 16, 17, and 18, in which quinaldic acid was found in significant amount only in eluates from a bed of A-162 that was incompletely bathed in eluant because it was free to float in

the solvent. If this resin floated when dehydrated with alcohol, some of the quinaldic acid may have remained to be eluted and detected in the experimental eluant. Obviously, if this hypothesis is correct, collection, concentration, and analysis of the dehydrating and conditioning solvents used in the standard protocol will recover the quinaldic acid. However, this prediction has not been tested experimentally.

The behavior of glycine in the parfait method also poses some questions. Table 21 shows the recovery of glycine from both non-standard and standard parfait columns in which it was tested. In all but the last column described, the glycine was quantified as its fluorescamine derivative. In columns prior to number 104, no blank control columns were prepared, and all fluorescamine-active material is reported as glycine. From columns 104 onward, control columns were processed in parallel using unspiked synthetic hard water, and the values reported as glycine represent the difference in fluorescamine-active material in corresponding fractions from spiked and unspiked columns. Control columns run with columns 104-107 showed no appreciable fluorescamine-active material in the column effluent, but color produced by the blank column's MSC-1 eluates corresponded to the recovery of about 50 µg (12%) of the glycine applied. In column 110, humic acid was also present and the apparent recovery of glycine in the column effluent here may actually represent differences in color produced by humic material reacting with fluorescamine.

The most revealing results were for columns 106 and 110, in which water or ammonium acetate was used to elute the MSC-1 bed after the standard parfait elution had been performed. In column 110, an additional 10% of the applied glycine was recovered, suggesting that a substantial amount of the glycine was still present unrecovered on this bed.

In column 110, it is also theoretically possible that glycine complexed with the added humic acid and that it was sequestered in the aqueous phase from the Teflon eluate and bound to the Teflon bed. To test this explanation, a 1/10-scale parfait column was constructed and 4 µCi of ^{14}C-glycine, 40 µg total, was applied in 800 ml of synthetic hard water (column 123). In this experiment, the alcohol and solvent 1 conditioning washes were combined with the standard eluates of each bed before counting. These solutions were not concentrated before counting. Quench correction was by the channels ratio method.

The data showed almost no recovery of glycine in the eluates. Furthermore, only 10.7% of the glycine could be accounted for on the Teflon and MSC-1 beds after elution. However, the data for the MSC-1 and Teflon beds are only minimum values because the correction for quench by the channels ratio methods doesn't work in heterogeneous systems. To estimate the actual amount of glycine on the MSC-1 resin, decreasing weights of the resin were counted, the apparent efficiency from the channels ratio was used to quantify the isotope detected, and

TABLE 21. RECOVERY OF GLYCINE ON PARFAIT COLUMNS

Column no.	Adsorbent: eluant[a]	Percent recovered[b]
28, 29, 31[c]	Teflon: diethyl ether	TR, TR, TR
	AG MP-50: 95% methanol;	TR, TR, TR
	1 M ammonia in 95% methanol	11, 10, 14
	1% ammonium acetate in 95% methanol	3, 1, 3
	AG MP-1: 95% methanol	TR, TR, TR
	1 M acetic acid in 95% methanol;	TR, 2, 2
	1% ammonium acetate in 95% methanol	2, 2, 3
	Effluent	14, 23, TR
	Total	30, 38, 22
67, 68, 70[c]	Teflon: methylene chloride	TR, TR, ND
	MSC-1: 1 M ammonium hydroxide;	43, 19, 21
	acetone then methylene chloride	TR, 2, ND
	IRA 400: 3 M ammonium hydrate;	TR, NF, ND
	acetone then methylene chloride	TR, TR, ND
	Effluent	4, NF, 1
	Total	47, 21, 22
104, 107	F2	5.9, 9.0
	F7	NF, NF
	Total	5.9 9.0
106	F2	13.1
	F2: additional 4 bed volumes of water	2.7
	F7	NF
	Total	15.8
110	F2	17.0
	F2: additional 5 bed volumes 1 M ammonium acetate	10.0
	F7	6.5
	Total	33.5
123[d]	F1	0.052
	F2	0.289
	F3-6, total	0.835
	F7	0.130
	Teflon	1.51
	MSC-1	9.21
	A-162	0.001
	Total	12.027

[a] F1-F7 are fractions from the standard parfait column.

[b] TR = trace; ND = not done; NF = not found. Entries correspond to each respective column number.

[c] Non-standard parfait columns.

[d] One-tenth-scale standard parfait column using ^{14}C-glycine. Eluates and drying/conditioning washes from individual ion exchange resins were pooled; entries for Teflon, MSC-1, and A-162 denote radioactivity on these beds after elution (see the text).

the percentage of the applied isotope calculated. As the weight of resin counted was decreased from 786 to 13 mg, the observed counts per weight of resin rose sharply, and the calculated amount of glycine detected rose from 1.3 to 9.2% of the isotope applied. This increase in counts per milligram of resin was not a simple function, complicating attempts to estimate the limiting counts per unit weight. Therefore, the reported yield of 9.21% of the applied isotope, which is the value calculated from the counting of 13 mg of MSC-1, should be considered a minimum value.

Although a mass balance for glycine was not achieved, several conclusions can be drawn from these experiments. First, the parfait column removed glycine essentially quantitatively from the water (0.13% of the isotope was recovered in the column effluent). Second, the glycine was bound strongly to the cation exchange bed and was eluted poorly by the standard protocol, as evidenced by the results from columns 106, 110, and 123. The results from columns 28-31 and 67-70 are difficult to interpret because of the possibility that material eluting from the column beds reacted with fluorescamine. Also, these experiments shed little light on the possibility that glycine reacts with humic acids, which are known to bind glycine (Lytle and Perdue, 1981). The only column run with humic acid, no. 110, showed the familiar pattern of partial recovery of glycine from the cation exchange bed, with a small additional recovery in the column effluent. If the effluent value was genuine glycine, then the humic acid may have been responsible for mobilizing the glycine to the effluent. In the control column for this experiment, however, the background color was half that in the spiked column. The difference in these values was the basis for the glycine reported in the table. The validity of the result rests upon the reproducibility of the humic acid level in the effluents in these columns. However since only a few percent of the humate applied appeared in the effluent, high reproducibility of humate levels seems unlikely.

Recovery of the Other Test Solutes on Standard Parfait Columns

The recovery of the other test solutes are reported in the data from columns 102-122 (Tables 19, 20, and 22). These were the only columns using the standard parfait protocol or small departures from the standard protocol, noted on the tables and below. Other than the suite of solutes tested on each column, the columns differed as follows: columns 102-109 used a 50-ml Teflon bed, columns 110-120 used a 150-ml bed, columns 109-120 included 2.0 ppm humic acid in the influent, and column 102 used eight bed volumes of methanol for fraction 5, rather than the usual four bed volumes. Columns 117, 118, and 120 were replicate runs including 16 test solutes at once, excluding only those solutes that could not be analyzed reliably in the presence of the solutes included. Column 119 was an unspiked control column run with humic acid and used to identify contaminants in the eluates of 117, 118, and 120. All seven fractions from these columns were analyzed by gas

TABLE 22. PERCENT OF RECOVERY of PHENANTHRENE and 5-CHLOROURACIL

	Phenanthrene	5-Chlorouracil
F1	98.8	3.0
F2	NF[a]	15.8
F7	1.2	10.0
Total	100	28.8

[a] NF = not found.

chromatography after methylation. If a chromatographable compound was not detected in a fraction from these columns, it is not reported. Only fraction 7, the vacuum distillate of the column effluent, was analyzed by the glucose oxidase method.

The porous Teflon bed gave reasonably reproducible recoveries of test solutes. Table 19 identifies the solutes found exclusively on Teflon and shows their recoveries. The influence of increasing the Teflon bed volume from 50 to 150 ml was mentioned previously. From column 106, it is evident that BHT and stearic acid were the two most strongly adsorbed solutes. The least well recovered solutes in this group were 1-chlorododecane and methyl isobutyl ketone; however, both of these solutes are so readily volatilized that losses during concentration of eluates must be considered a likely source of their low recovery.

Glucose was recovered quantitatively in the column effluent, whether humic acid was present or absent. Presumably, all other poorly volatile, neutral hydrophilic solutes would also be recovered in this fraction.

Among compounds that adsorbed to Teflon and to at least one other bed, isophorone, caffeine, furfural, phenanthrene, and 2,4-dichlorophenol must be mentioned. Isophorone and phenanthrene were recovered essentially only from Teflon, minor amounts being found in other fractions. The finding of phenanthrene in F7 must be considered provisional, because its assay was by fluorescence, and the value reported was based on a very small signal difference between the F7 of the control and experimental columns. Caffeine was adsorbed to Teflon and the cation exchange bed and was found in the column effluent. As previously mentioned, caffeine's recovery was decreased slightly when humic acid was included (column 109 compared with 108), and decreased strikingly when the larger Teflon bed and 15 other solutes were included (columns 117, 118, and 120). In the latter group of columns, caffeine was no longer recovered from Teflon; its absence from fraction 7 of

column 120 is unexplained and is inconsistent with the previous four trials reported in the table.

Three compounds recovered from parfait columns were also previously tested for breakthrough from 5-ml Teflon beds. The capacity factors for these compounds and their recoveries from the Teflon bed of a parfait column showed a rough correlation. Phenanthrene, which was tested in the parfait column only in the presence of humate, was recovered essentially quantitatively from Teflon and had a capacity factor of 368. About 15% of the caffeine applied to a parfait column in the absence of humate could be recovered from Teflon, and caffeine showed a capacity factor of 22. Only about 2% of the 2,4-dichlorophenol applied to parfait columns could be recovered on Teflon; its capacity factor was 5.6. It may therefore be anticipated that compounds following the inverse correlation of solubility with capacity factor and having a capacity factor greater than about 20 should be detectably adsorbed to the Teflon bed of a parfait column. Simply increasing the volume of the Teflon bed may also increase the absolute recovery of adsorbable solutes that have modest values of k′. For this reason, a 150-ml bed of Teflon per 8 l of water may not be the ideal bed size; a larger bed may be better.

Unrecovered Solutes

Although not all of the applied solutes were recovered in parfait column eluates, reasonable suggestions can be made about the locations of the missing compounds. For example, the unrecovered methyl isobutyl ketone, 1-chlorododecane, and chlorobiphenyls were surely lost from the F-1 eluate by vaporization during concentration.

Trimesic acid, on the other hand, appeared to be so strongly adsorbed to the anion exchange resin that it was incompletely recovered in the eluate. As previously discussed, glycine also appeared to bind tightly and to elute incompletely by the standard protocol. Both of these compounds probably could be eluted with aqueous solutions of high ionic strength, but this would create the problem of recovering the solute from the eluate. Volatile buffers may offer a solution to that problem.

Isophorone, 5-chlorouracil, and quinoline were probably lost by vaporization from the column effluent, F7. Each of these solutes is sufficiently water soluble not to adsorb to Teflon, but each is volatile enough to be lost during the vacuum concentration of F7. Recall that only 20% of 15 mg of quinoline was recovered during lyophilization of a solution to half of its original volume (Table 4). Isophorone and 5-chlorouracil were detected in F7 and at least part of the unrecovered fraction of each of these componds must have been lost during the vacuum concentration of this effluent. (Eluates of the anion exchange bed were not analyzed for 5-chlorouracil which thus could also have been present on this bed; see Table 22.) Quinoline was not detected in F7 but its

water solubility and weak basicity suggest that any of it that escaped the cation bed would have gone to the effluent, where it would have vaporized.

Furfural is such a reactive, volatile, and water-soluble compound that its loss could be due to several causes. Oxidation and volatilization from eluates, especially from the column effluent, are the most likely sources of loss.

Humic acid seemed to affect the recovery of two solutes, caffeine and 2,4-dichlorophenol. These are discussed in the section on humic acid (above).

Behavior of Hypochlorite

The influence of 2 ppm hypochlorite was assessed in an unspiked control column. Each eluate was assayed for hypochlorite using the ferrous N,N-diethyl-p-phenylenediamine titrimetric method (Franson, 1976). No hypochlorite was detected. Each eluate was also analyzed by gas chromatography and found to be virtually identical to a blank column without hypochlorite run simultaneously.

FUTURE USE OF THE PARFAIT METHOD

The object of this study was to understand the behavior of a broad range of chemical types in this recovery method. Based on this evaluation, and on similar work with other recovery methods, a synthesis of methods is to be proposed that would provide a comprehensive recovery of chemical contaminants in water.

The study of the parfait method reported here shows that it does not recover all classes of trace contaminants in water with equal efficiency. Volatile compounds are readily lost in this method. Contamination of eluates during elution of the ion exchange beds is also a major problem. Even if this contamination were acceptable, the elution of these beds is not complete, as is illustrated by the behavior of trimesic acid and glycine. Porous Teflon on the other hand offers a means to quantitatively and cleanly recover a set of water contaminants, albeit a set that was not the primary objective of the original parfait method.

Strengths of the method include its ability to quantitatively recover neutral, nonvolatile solutes like glucose and its exceptional recovery of aromatic cations and humic acids on Chromosorb T. It would be interesting to see if porous Teflon would recover aromatic cations like paraquat and diquat from environmental media. Also, the generality of humate adsorption needs to be tested using a range of humic and fulvic acid samples.

The original concept of the parfait method was to use vacuum distillation to concentrate the nonvolatile contaminants in water. The complicating factors in the current modified parfait method come mainly from the ion exchange beds, which are necessary to remove the metal cations from water so that intractable precipitates will not form during vacuum concentration. The intractable nature of such precipitates is believed to come from the interaction of metal carbonates with lipids and humic substances during concentration. Presumably the intractability of these precipitates would be reduced or abolished by removing the lipids and humics before concentration, rather than removing the metals by ion exchange. This study shows that porous Teflon will remove of these materials, suggesting that vacuum concentration might still be used as a comprehensive method to concentrate water-soluble, nonvolatile compounds if a water sample were first passed over a large bed of porous Teflon. The bed would probably have to be larger than the 150 ml used here, so that essentially all of the humic material would be removed.

The success of this method depends upon the generality of Teflon's removal of humic acids. Teflon has been tested only with the Fluka humic acid provided by HERL. Also, the prediction that a humic- and lipid-free effluent will form residues that redissolve under mild conditions must be tested. Furthermore, problems may be encountered in scaling up the Teflon bed. For instance, if large samples are not degassed before passage over the bed, dissolved air tends to accumulate and cause some Teflon particles to float.

The proposal above has advantages: porous PTFE does not contribute discernable contaminants to its eluates; hydrophobic, Teflon-adsorbable solutes can potentially be recovered in organic solvents; and humate can be recovered from Teflon with suitable aqueous eluants. This approach should be given a trial by testing the recovery of the model solutes used in this study and by testing the formation and resolubilization of precipitates obtained from a few natural polluted waters.

REFERENCES

Bacaloni, A., G. Goretti, A. Lagana, B.M. Petronio and M. Rotatori. 1980. "Sorption Capacities of Graphitized Carbon Black in Determination of Chlorinate Pesticide Traces in Water." Anal. Chem. 52:2033-36.

Bikales, N.M., Ed. 1970. "Encyclopedia of Polymer Science and Technology," Vol. 13, John Wiley and Sons, New York, pp. 623-654.

Burns, K.A. and J.L. Smith. 1980. "Hydrocarbons in Victorian Coastal Waters." Aust. J. Mar. Freshwater Res. 31:251-56.

Carter, C.W. and I.H. Suffet. 1982. "Binding of DDT to Dissolved Humic Materials." Environ. Sci. Technol. 16:735-740

Cesaro, A., E. Russo and D. Tessarotto. 1980. "Thermodynamics of Caffeine in Aqueous Denaturant Solutions." J. Solution Chem. 9:221-235.

Coppola, E.D. and J.G. Hanna. 1976. "Simple Fluorimetric Analysis of Glycine in Dietetic Beverages." J. Chem. Ed. 53:322-23.

Fales, F.W. 1963. "Glucose (enzymatic)" in Standard Methods of Clinical Chemistry, Vol 4, D. Seligson, Ed. (Academic Press: New York) pp. 101-111.

Franson, M.A., Ed. 1976. "Determination of Inorganic Nonmettalic Constituents: Chlorine (residual)" in Standard Methods for the Examination of Water and Wastewater 14th Ed (Am. Public Hlth. Assoc.: Washington, D.C.) pp. 329-332.

Gangal, S.V. 1980. "Polytetrafluoroethylene" in the Kirk-Othmer Encyclopedia of Chemical Technology Vol. VII, 3rd Ed. (John Wiley and Sons: New York) pp. 1-24.

Gehrke, C.W., D.L. Stalling, and C.D. Ruyle. 1967. "Quantitative Gas-Liquid Chromatography of TMS Nucleic Acid Constituents." Biochem. Biophys. Res. Commun. 28:869-74.

Guttman, D. and T. Higuchi. 1957. "Reversible Association of Caffeine and Some Caffeine Homologs in Aqueous Solution." J. Am. Pharm. Assoc. 46:4-10.

Hansch, C., A. Leo. 1979. "Substituent Constants for Correlation Analysis in Chemistry and Biology," John Wiley and Sons, New York.

Jahangir, L.M. and O. Samuelson. 1980. "Chromatography in Aqueous Solution on Styrene-Divinyl Benzene Resins." J. Chromatogr. 193:197-207.

Johnston, J.B. and M.K. Verdeyen. 1981. "Recovery of Mutagens from Water by the Parfait/Distillation Method" in Chemistry in Water Reuse, Vol 2, W.J. Cooper, Ed. (Ann Arbor Science Press: Ann Arbor, MI) pp. 171-189.

Junk, G.A., J.J. Richard, M.D. Grieser, D. Witiak, J.L. Witiak, M.D. Arguello, R. Vick, H.J. Svec, J.S. Fritz and G.V. Calder. 1974. "Use of Macroreticular Resins in the Analysis of Water for Trace Organic Contaminants." J. Chromatogr. 99:745-62.

Kawabata, N. and K. Ohira. 1979. "Removal and Recovery of Organic Pollutants from Aquatic Environment. 1. Vinylpyridine-Divinylbenzene Copolymer as a Polymeric Adsorbent for Removal and Recovery of Phenol from Aqueous Solution." Environ. Sci. Technol. 13:1396-1402.

Keith, L.H. 1977. "GC/MS Analyses of Organic Compounds in Treated Kraft Paper Mill Wastewaters" in Identification and Analysis of Organic Pollutants in Water, L.H. Keith, Ed. (Ann Arbor Science Press: Ann Arbor, MI) pp. 671-707.

Kucera, P., S.A. Moros and A.R. Mlodozeniec. 1981. "Differential Frontal Analysis of Carboxylic Acids." J. Chromatogr. 210:373-88.

Lagana, A., B.M. Petronio and M. Rotatori. 1980. "Concentration and Determination of Polycyclic Aromatic Hydrocarbons in Aqueous Samples on Graphitized Carbon Black." J. Chromatogr. 198:143-49.

Leenheer, J. 1981. "Comprehensive Approach to Preparative Isolation and Fractionation of Dissolved Organic Carbon from Natural Waters and Wastewaters." Environ. Sci. Technol. 15:578-87.

Leoni, V., G. Puccetti, R.J. Colombo and A.M. D'Ovidio. 1976. "The Use of Tenax for the Extraction of Pesticides and Polychlorinated Biphenyls from Water." J. Chromatogr. 125:399-407.

Locke, D. 1974. "Selectivity in Reverse-Phase Liquid Chromatography using Chemically Bonded Stationary Phases." J. Chromatogr. Sci. 12:433-37.

Lytle, C.R. and E.M. Perdue. 1981. "Free, Proteinaceous and Humic-Bound Amino Acids in River Water Containing High Concentrations of Aquatic Humus." Environ. Sci. Technol. 15:224-228.

MacGee, J. 1966. "Determination of Nucleic Acid Base Ratios by Gas-Liquid Chromatography" Anal. Biochem. 14:305-14.

MacKay, D., A. Bobra and W.Y. Shiu. 1980. "Relationship Between Aqueous Solubility and Octanol-Water Partition Coefficients." Chemosphere 9:701-11.

Matsuda, K. and M. Schnitzer. 1971. "Reactions Between Fulvic Acid, a Soil Humic Material, and Dialkyl Phthalates." Bull. Environ. Contam. Toxicol. 6:200-204.

May, W.E., S.P. Waslk and D.H. Freeman. 1978. "Determination of the Solubility Behavior of Some Polycyclic Aromatic Hydrocarbons in Water." Anal. Chem. 50:997-1000.

Navratil, J.D. 1977. "Open-Pore Polyurethane Columns for Collection and Preconcentration of Polynuclear Aromatic Hydrocarbons from Water." Anal. Chem. 49:2260-63.

Otsuki, A. and R.G, Wetzel. 1973. "Interaction of Yellow Organic Acids with Calcium Carbonate in Freshwater." Limnol. Oceanogr. 18:490-93.

Picer, N. and M. Picer. 1980. "Evaluation of Macroreticular Resins for the Determination of Low Concentrations of Chlorinated Hydrocarbons in Sea Water and Tap Water." J. Chromatogr. 193:-357-69.

Robb, E.W. and J.J. Westbrook III. 1963. Preparation of Methyl Esters for Gas Chromatography of Acids by Pyrolysis of Tetramethylammonium Salts." Anal. Chem. 35:1644-47.

Rosen, A.A. 1977. "The Foundations of Organic Pollutant Analysis" in Identification and Analysis of Organic Pollutants in Water, L.H. Keith, Ed. (Ann Arbor Science Press: Ann Arbor, MI) pp. 3-14.

Shackelford, W.M. and L.H. Keith. 1976. "Frequency of Organic Chemicals Identified in Water" USEPA Report 600/4-76-062, Environmental Research Laboratory, Athens, GA.

Sigma Chemical Co. 1979. "The Enzymatic Colorimetric Determination of Glucose." Technical Bulletin No. 510 (Sigma: St. Louis, Mo).

Suess, E. 1970. "Interaction of Organic Compounds with Calcium Carbonate - I. Association Phenomena and Geochemical Implications." Geochim. Cosmochim. Acta 34:157-68.

Thon, N., Ed. 1941. "Annual Tables of Physical Contants and Numerical Data," Am. Committee on Annual Tables, Princton, N.J., Table 700[c].

Thurman, E.M., R.L. Malcolm and G.R. Aiken. 1978. "Prediction of Capacity Factor for Aqueous Organic Solutes Adsorbed on a Porous Acrylic Resin." Anal. Chem. 50:775-779.

Washko, M.E. and E.W. Rice. 1959. "Determination of Glucose by an Improved Enzymatic Procedure." Clin. Chem. 7:542-45.

Yano, Y. and M. Janado. 1980. "Hydrophobic Interaction Chromatography of Aliphatic Alcohols on Unsubstituted Sephadex Gels with High Dextran Concentrations." J. Chromatogr. 200:125-36.